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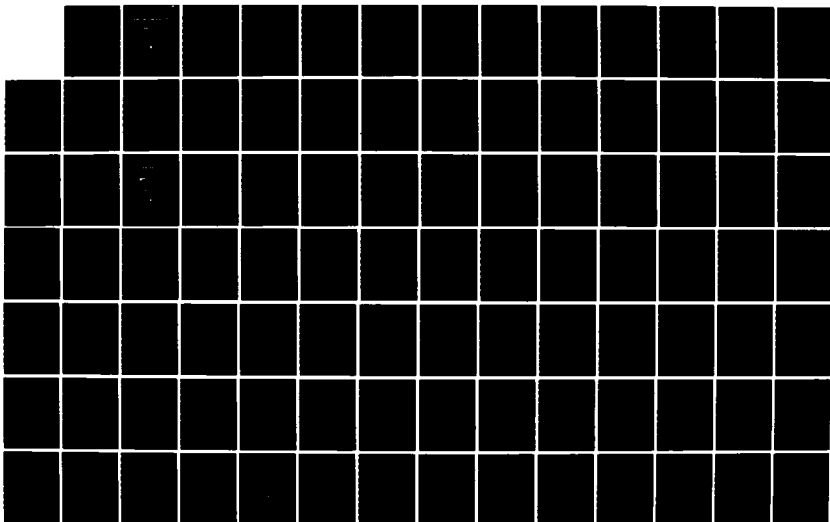
OCEANIC CHEMISTRY AND BIOLOGY GROUP (ONR CODE 422CB)
PROGRAM SCIENCE REPORT FY 81(U) OFFICE OF NAVAL
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OCEANIC CHEMISTRY AND BIOLOGY GROUP

(ONR CODE 422CB)

PROGRAM SCIENCE REPORT

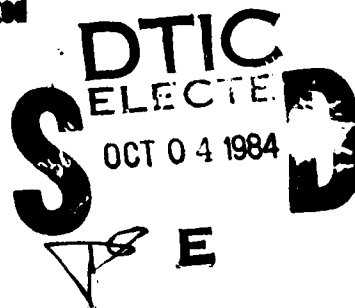
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Dissolved Organics
HEBBLE
Marine Biology
Marine Chemistry
Marine Fouling
Marine Mammals
Noxious Marine Organisms
Oceanic Biology
Oceanic Chemistry

Oceanic Mixing
Particulate Matter
Sediment Traps
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Trace Elements
Trace Metals
Underwater Visibility
Underway Chemistry
Water Mass Characteristics

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OCEANIC CHEMISTRY AND BIOLOGY GROUP

(ONR CODE 422CB)

PROGRAM SCIENCE REPORT FOR FY 1981

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OFFICE OF NAVAL RESEARCH

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SECTION I

INTRODUCTION

The recent amalgamation of the Chemical Oceanography and Oceanic Biology Programs into the Oceanic Chemistry and Biology Group presents its first joint report of our current contract research science effort. It is a compilation of those research efforts currently being funded by Code 422CB, written by the individual Principal Investigators. It represents how we view our program objectives in terms of science, and it is a measure of our program effectiveness.

→ The objectives of these programs is to further our understanding of the chemical and biological processes which control the abundance and distributions of chemical and biological species in the ocean. These are fundamental questions of basic research. From among the class of forefront problems which may be addressed we tend to focus on those which can be perceived as having high potential for impact on present or future Navy operations. ↙

Although the Code 422CB programs are mission oriented, research efforts must be, first and foremost, of fundamental scientific importance and the results of these efforts must appear in the open and refereed scientific literature. The scientific progress that appears in the literature is a tangible product of our support and is a measure of our program effectiveness. A glance at the substantial bibliography produced in the past two years by program investigators reveals that our contractors are among the most productive in their scientific community.

We appreciate the efforts of all the P.I.'s whose work makes this volume possible and who continually help us with advice, briefing materials and tutoring. Frequently ONR scientific officers must serve as surrogate investigators in defending scientific efforts and in competing for finite resources. To the end that we are successful the assistance and backing by our P.I.'s helps us all.

REORGANIZATION OF
THE CONTRACT RESEARCH PROGRAM
OF THE OFFICE OF NAVAL RESEARCH

On 29 June 1981, the contract research program of ONR (Code 400) was reorganized into four directorates: Mathematical and Physical Sciences, Code 410; Environmental Sciences, Code 420; Engineering Sciences, Code 430; and Life Sciences, Code 440. The old Ocean Science and Technology Division, Code 480, is no more; it is now part of the Environmental Sciences Directorate which contains two Divisions: Ocean Sciences, Code 422; and Geophysical Sciences, Code 425.

Each Division is organized into several programs which remain essentially unchanged in structure from the previous organization. Two modifications of programs involve terrestrial geology and marine geology and geophysics which have been merged into one group in a new Division, and chemical oceanography and oceanic biology which have been merged into one group, but which remain in the Ocean Sciences Division. The unofficial organization diagrams following will help to make clear the new program structure. It is hoped that the restructuring will facilitate cross-disciplinary interactions between the environmental sciences programs. In particular, we hope that interdisciplinary marine science will thrive under this new management.

For those of you who deal either with the former programs in Chemical Oceanography (formerly Code 482) or Oceanic Biology (formerly Code 484), the Scientific Officers, their addresses and telephone numbers remain essentially unchanged. For work which is principally chemical, contact:

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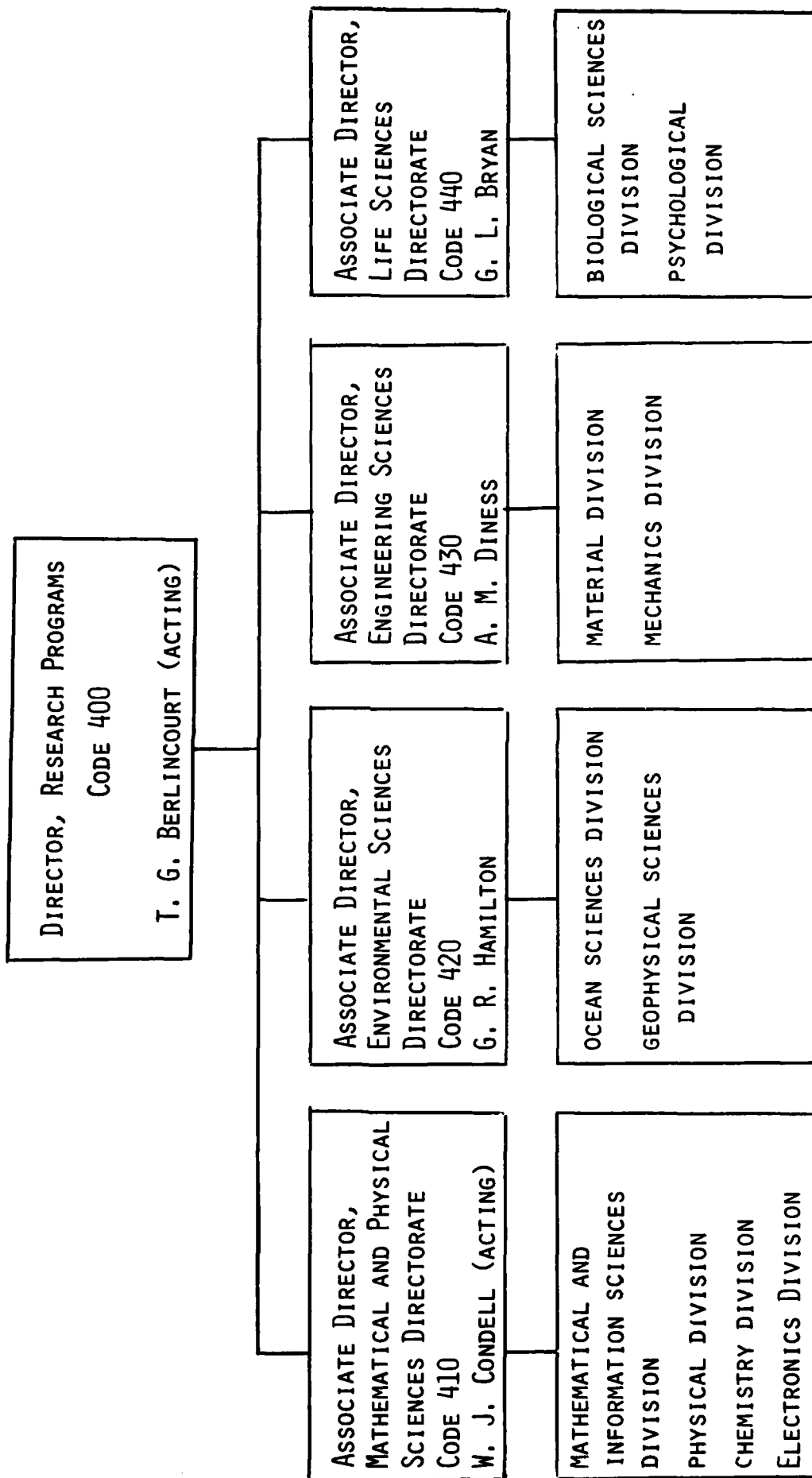
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ARCTIC RESEARCH GROUP

GROUP LEADER: G. L. JOHNSON

ONR CONTRACT RESEARCH PROGRAM



SECTION II

AREA AND PROJECT SUMMARIES

OCEANIC BIOLOGY AREA SUMMARIES

Marine Biodeterioration

The following abstracts and a major symposium sponsored by ONR on this topic early in 1981, reveal that the focus of this program has changed dramatically over the last decade. Both the theory of ecological succession and pragmatic considerations of fouling control have drive attention toward initial fouling events: dynamics of microbial adhesion, cues for larval settlement, and triggers of metamorphosis. Investigative techniques range from hydraulic flumes to recombinant DNA technologies.

The genuinely exciting findings summarized in our program abstracts suggest that this focus on initial fouling mechanisms and processes continues to be amply rewarded. Providing a desirable balance with respect to this current thrust is a continuing, if lower-level, commitment to a limited number of investigations of basic biology of the more poorly known groups of foulers and borers.

Upper Ocean Biomass Structure

Emphasis of this program continues to be on the study of the open ocean ecosystem, but focuses on the development of sampling techniques that are capable of sampling at spatial scales and rates appropriate to physical processes which are likely to influence biological patchiness. Several of these gear development efforts are reaching fruition nearly synchronously, and we expect their application to a number of interesting biological, physical, and chemical oceanographic questions on small spatial scales (below mesoscale) in the next several years. The long-term goal of this effort is to achieve a predictive capability with respect to organism spatial distribution on these small scales, where physics and behavior interact strongly.

One subelement of this program focuses on gaining a fundamental understanding of mechanisms and characteristics of marine bioluminescence. Component studies include investigations of the physiologies, distributions and ecologies of causative organisms.

II-04

DISSERTATIONS SYMPOSIUM IN CHEMICAL OCEANOGRAPHY

(DISCO)

Long Range Scientific Objectives

A continuing concern to the Office of Naval Research, the National Science Foundation, and the National Oceanic and Atmospheric Administration is the effective utilization of young scientists. We desire to know what is happening to new graduates from our universities, and to nurture their enthusiasm. We also wish to inform them about the research climate and opportunities in their respective fields. Therefore, we were extremely receptive to a suggestion made in October 1976 by Dr. E. D. Goldberg of the Scripps Institution of Oceanography that it would be useful to have a symposium convened which would be completely dedicated to soon-to-be or recent Ph.D. graduates. After lengthy discussions with members of the scientific community preparations were begun for such meetings which were entitled "Dissertations Symposium in Chemical Oceanography" (DISCO).

Current Status of the Project

During the week of February 20-25, 1981, the third of these research symposia for new Ph.D.'s in chemical oceanography was convened at the University of Hawaii's East-West Center. The meeting was jointly sponsored by the Office of Naval Research (ONR), the National Science Foundation (NSF), and the National Oceanic and Atmospheric Administration (NOAA) with coordination by the American Institute of Biological Sciences. The symposium provided scientific discussion and interaction among chemical oceanography doctoral students who have received or who will receive their degrees.

Significant Accomplishments

We believe that professional relationships were forged at this meeting that will facilitate future interdisciplinary and inter-institutional investigations. We also hope that the entire oceanographic community will become better aware of innovations in marine chemistry as a consequence of making young worker's efforts more visible through such a symposium. Time was provided for extended informal discussion among members of the symposium. Another purpose of the meeting was to provide the attendees with information on the composition of the Federal program in ocean sciences, and to be appraised of where we perceived the field of chemical oceanography to be going. Attendees also were provided with the opportunity to express their views in this regard. One-half day of the meeting was devoted to meet this objective, as well as time and facilities being provided to the participants to develop specific research directions for the future.

In reviewing the symposium contributions several generalizations can be made. Both the contents of the sessions and the extensive discussions of the symposium's informal sessions suggest that chemical oceanographers are continuing to stress the need for greater interdisciplinary interaction in unraveling the forefront problems of marine chemistry. The most obvious and most immediately rewarding symbiosis is in marine organic chemistry and marine microbiology. Specifically, chemists need information on microbial activity and specific microbial transformations of organic substances in order to interpret data on distributions of organic compounds and other species in the nutrient cycle. Microbiologists would benefit from better information on substrates available to and being metabolized by microorganisms. Another interdisciplinary area of continuing

importance is the marine geological-marine chemical field. Session V, "Chemical Processes in Sediments and Particulates", was one of the largest parts of the symposium and stimulated extensive discussion. The attendees felt that disciplinary hurdles were less of a problem to surmount in this area, possibly because academic marine programs have traditionally grown-up in conjunction with or as extensions of geochemistry programs. The young chemical oceanographers at this meeting called strongly for elimination of any artificial administrative barriers to funding of interdisciplinary and multidisciplinary proposals. We believe it is incumbent upon the funding agencies to heed this call lest we neglect those areas of oceanography that are on the very forefront of research and that potentially offer the most benefit for society as well as science.

The informal group discussion generated a number of specific recommendations for future research. A few of these recommendations are:

1. Technical centers with state-of-the-art equipment should be regularly maintained and made available both to experienced and unexperienced researchers. These probably could be expansions of present university facilities.
2. "Descriptive-type" research should be considered a low priority, and more emphasis placed on determining mechanisms. Additionally, there should be an effort made by the agencies to publish data or provide newsletters to acquaint other researchers of recently acquired data from reports.
3. More emphasis on research integrating basic trophic relationships with the turnover times of compounds in the ocean is needed. In particular, the movement of carbon from the primary producers to the consumers creates fluxes of dissolved organic compounds and particulates with influence the turnover of certain kinds of organic compounds and the scavenging of metals and other trace elements. The mechanism for these interactions must be studied with less emphasis on descriptive work.
4. Interactions between workers in the fields of marine organic chemistry and marine microbiology should be encouraged. One way of accomplishing this would be by organizing a meeting on the subject of "microbial transformations of organic matter in the marine environment". The purpose of this meeting should be to determine what specific problems are most worthy of joint study and to establish contacts and make preliminary plans for interdisciplinary research.
5. A mechanism for encouraging the submission and funding of joint proposals in the area of marine organic chemistry/microbiology need to be established.

Future Plans

We consider the symposium to have been extremely successful and look forward to supporting future sessions. In fact, we believe it would be useful to have this type of meeting for all the individual disciplines which combine to form the ocean sciences, and in some cases, possibly meeting in parallel sessions, where appropriate. Such a consideration will be entertained when planning for future meetings. Our application data since 1978 indicate that the U.S. produces approximately 20 Ph.D. chemical oceanographers per year. Thus an interval of eighteen months between meetings yields an optimum number of participants. The next meeting, DISCO IV, is planned for October 1982.

BIOLOGICAL SUPPORT OF HIGH ENERGY BENTHIC BOUNDARY LAYER EXPERIMENT (HEBBLE)

Although oceanic biology as an environmental science is by its very nature inter- or multi-disciplinary, some of the program's biological studies are addressed explicitly toward answering questions whose primary thrusts are not biological. The major such effort is in support of the High Energy Benthic Boundary Layer Experiment (HEBBLE), and is a joint effort with the Marine Geology and Geophysics Program and the Physical Oceanography Program.

The scientific goal of HEBBLE is to describe the genesis of the bedforms found in cohesive sediments in the deep ocean and to predict their rate of movement. Such a goal requires that the near-boundary flow responsible for the shaping of the bed be measured precisely and that models of the boundary layer be developed to include the effect of stratification due to suspended sediment. Manipulative field experiments and laboratory studies to evaluate the influence of organism activity on sediment properties including geotechnical parameters and the resultant effects on critical shear stress must be carried out in order that the boundary layer model includes a realistic sediment transport model. Long-term experiments with a main lander and out stations to measure the characteristics of the total Ekman layer and short-term experiments with a small BASS frame to measure the flow in the logarithmic region must be carried out in order to measure the scales of variability of the stress, velocity and sediment flux fields so that the time-dependent structure of the flow may be modeled.

There are, therefore, two major themes in HEBBLE: flow-boundary interactions which requires that the critical erosion velocities be measured and that the momentum field in the boundary be known for input to a turbulent Ekman layer model; and sediment flux studies which also require a precise model for the vertical flux of materials.

Methodology

The long-term goal of HEBBLE is to describe the genesis of bedforms found in cohesive sediments in the deep ocean and to predict their rate of movement. To make progress towards this goal and in understanding the transport of sediment in the deep ocean, a series of experiments, coupled with laboratory modeling and extensive geological and biological taxonomic work, has been proposed; such work will be carried out in concert with the modeling of the benthic boundary layer and its sediment transport.

The problem of the prediction of sediment transport in the deep ocean is shared by physical oceanographers concerned with ocean mixing and the diffusion of scalars; by biologists who are attempting to model the foraging strategies of soft-bottom benthic communities; by geomorphologists trying to describe the bedforms and their mode of formation in the deep ocean; and by sedimentologists who wish to infer from the bedforms the paleo-currents. Most work on marine sediment transport in the past has been equivocal: observation and measurement have not usually been in strong accord with prediction. However, this disparity is often due to the operation of

intervening variables not explicitly included in the predictive model (non-uniformity in the momentum field; biogenic stabilization or destabilization of sediments; dynamical influences of suspended sediment not properly incorporated in a model, inadequate characterization of the hydrodynamic properties of the sediments) or to uncertainty in the accuracy of the data (averaging times too short being the most common problem).

The immediate goal of HEBBLE is the development of a model and its field verification to predict sediment transport in areas of strong bottom boundary layer flows in the deep ocean. The inputs will be the sediment characteristics, both biological and physical, the flow field, and the boundary topography. Geologists and physical oceanographers within HEBBLE are placing initial focus on an area of the lower Scotian rise between 4800m and 4900m, where they have documented near-bottom currents frequently in excess of 30cm sec^{-1} and occasionally in excess of 70cm sec^{-1} . Longitudinal ripples are frequent at these depths, often appear to form first as "comet marks" behind biologically produced mounds, and may be the result of bedload transport of biologically pelletized sediments. As a consequence, the primary focus of the biological component of HEBBLE is on the modification of sediment transport parameters by organisms.

The shared nature of the problem offers some unique opportunities. Geotechnical measurements, long presented as an appendix to work on cohesive sediments, can be integrated with the biological research on mucal polysaccharide concentrations. Textural measures routinely obtained by geologists may be examined in the light of biological studies of particle selection by deposit feeders and pelletization.

Materials exposed in the ocean undergo deterioration by the combined action of chemical corrosion, biological fouling and physical erosion. Most often, the observed effects result from a combination of all three processes. From a chemical oceanographic viewpoint, a number of factors are important in influencing corrosion; the electrode potential of a metal in seawater; the nature and concentrations of dissolved gases, such as oxygen, carbon dioxide, and hydrogen sulfide; the nature and concentrations of dissolved organic material near the corroding surface; local changes in pH; changes in redox potentials in going from oxidizing to reducing environments; and others. Although this list is incomplete, it does serve to indicate the complexity of the problem encountered and the requirement for a better understanding of fundamental chemical processes in the ocean environment to combat corrosion from a sound scientific basis.

Yet another consideration on the behavior of materials in the sea is the problem of biological fouling. The relationship between chemical corrosion and fouling is most intimate, but as yet not well understood. Although a solution to this problem will require the combined efforts of the chemical oceanographer and marine biologist, it must be emphasized that an understanding of the chemical oceanographic characteristics of the anti-fouling agent employed is required to prevent degradation of the water quality of the body of water in which the material has been inserted (e.g. high levels of toxic trace elements being released to the medium).

Particulate Fluxes

1. What are the chemical characteristics of the geological and biogenic solids that exist in the water column?
2. How are these materials altered by adsorption and remineralization phenomena?
3. What are the temporal variations in these processes?
4. How does the sediment reaching the seafloor reflect the conditions of the overlying water column?

These questions serve as a basis for investigating particulate fluxes in the marine environment. It has become increasingly apparent that chemical reactions of settling particulates play a significant role in the recycling of many important elements through the euphotic zone. They undoubtedly are crucial in understanding the removal rates of many elements from the water column.

OCEANIC CHEMISTRY AREA SUMMARIES

Open Ocean Chemical Variability

For many years, the tools and techniques available to oceanographers made it almost impossible to study any other sort of phenomenon in the ocean except the long term average. We know, for example, that very large scales of motion, the general circulation, are set by very large phenomena, by the large scale wind stress pattern, by the large scale of solar heating and cooling, and by the broad bathymetry of the ocean basins. However, as physical oceanographers developed the techniques and tools to allow them to measure on finer grids and on shorter time scales -- tools such as the expendable BT, towed thermistor chains, profiling devices of various kinds, the use of aircraft for rapid synoptic surveys, and recently satellite imagery -- they found that the mesoscale fluctuations are much more energetic than the general circulation. We now know that the great bulk of the energy supply to the ocean does not drive the general circulation but rather drives the finer scale fluctuations in the ocean, with horizontal space scales of tens to several hundreds of kilometers, and time scales not of decades or years but of many days to several months.

One can make a rather good analogy between the way that physical oceanography has developed and the way the meteorology has developed. Climate is studied by looking at records accumulated over many years and extracting the broad, large scale length patterns, whereas to study weather synoptic maps are needed; one needs to look at the changing instantaneous patterns; one needs to look at the passage of fronts. It is not surprising that physical oceanographers have taken over meteorological terms such as "fronts" to describe the sort of phenomena to which they have turned their emphasis. But marine chemists are still largely confined to studying the chemical climate. We are not yet looking at the analogous "weather" of marine chemistry. It is largely so because we do not have the tools to allow us to look at the same time and space scales that the physical oceanographers have developed.

The ONR program area in chemical variability has an emphasis, therefore, on development of methods and instruments to provide the tools needed by the marine chemical community to address small scale length features and rapidly evolving phenomena. Underway analysis is stressed and continuous or semi-continuous measurement techniques are sought. The ultimate goal, however, is to address processes and mechanisms which control the distribution of chemical species in the oceans.

Stability of Materials and Compounds in the Ocean

The deterioration of materials in the marine environment is responsible for a significant national expenditure. A conservative estimate of the cost of corrosion and protection against corrosion in the United States is eight billion dollars per year. At the present time just the cost of the anti-fouling paint used by the Navy is on the order of fifty million dollars per year.

SECTION III

INDIVIDUAL PROJECT LISTINGS

SECTION III

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INDIVIDUAL PROJECTS BY STATE AND INSTITUTION

CALIFORNIA

University of California, Los Angeles

Wasson, J. T. and Kyte, F. T.: Extraterrestrial Noble Metals in Marine Sediments

University of California, Santa Barbara

Case, J. F.: Bioluminescence Mechanisms

Morse, D. E.: Biochemical Control of Marine Fouling

California State University, Hayward

Schusterman, R. J.: Mammal Underwater Acoustics

California State University, Long Beach

Nelson, D. R.: Field Investigations of Shark Behavior

Hubbs Sea World Research Institute

Evans, W. E.: The Biology of Small Whales

San Diego Natural History Museum

Shulenberger, E.: Patchiness in Oceanic Biology and Physics

Scripps Institution of Oceanography

Bada, J. L.: The Organic Chemistry of Sea Water: Combined Amino Acids, α -keto acids, and Dicarboxylic Acids

Goldberg, E. D.: The Marine Biogeochemistries of the Platinum Metals

McGowan, J. A.: Central Gyre Research Program

Mullin, M. M.: Investigation of Plankton Patchiness Relative to Hydrography and Biology

Nealson, K. H.: Mechanisms of Bioluminescence: Kinetics-Spectral and Biochemical Properties as a Probe of Marine Communities and Interactions

Smith, K. L., Jr.: Ecological Energetics of Deep Scattering Layer Animals:
In Situ Studies

Tiburon Center for Environmental Studies

Compagno, L. J. V.: Guide to Sharks of the World

Tracor, Inc.

Holliday, D. V.: Studies of Ocean Volume Reverberation at High Acoustic Frequencies

Naval Ocean Systems Center

Zirino, A.: Real-Time Marine Chemistry

Naval Postgraduate School

Haderlie, E. C.: Biology of Stone and Wood Borers in Monterey Submarine Canyon and Deeper Water off Central California

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University of Southern California

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SECTION IV

OCEANIC CHEMISTRY ABSTRACTS

PROGRESS REPORT ABSTRACTS



OCEANIC CHEMISTRY



1 MARCH 1982

**OFFICE OF NAVAL RESEARCH
ENVIRONMENTAL SCIENCES DIRECTORATE**

SECTION IV

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OPEN OCEAN CHEMICAL VARIABILITY

COPPER IN THE SURFACE WATERS OF
THE NORTH ATLANTIC

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Long-Range Scientific Objectives

Our long-range goal is to understand mechanisms influencing trace element distributions in ocean surface waters, in particular the causes of excess copper which is observed in certain regions of the ocean

Project Objectives

We are using current technology in limited studies of trace element distributions in a few regions to define potential sources of trace element anomalies. We are collaborating with Dr. David Reid (NORDA) in a comparison of the surface distributions of ^{228}Ra and Cu. We are developing rapid shipboard liquid chromatographic techniques for trace element analysis that will allow for more thorough studies in the future.

Current Status

During the current year, we are participating in 4 cruises. Two of these are undertaken in association with Dr. David Reid for ^{228}Ra - Cu intercomparison (Gulf of Mexico and the Gulf of California). Two other cruises are being undertaken separately to test our new non-contaminating trace element sampler. In addition, we are completing laboratory analyses on samples from 3 cruises from the previous year.

We have evaluated and purchased most of the components for our liquid chromatography system; these components are now being run through their paces separately in order to evaluate their specifications. We anticipate that the assembly into the chromatographic system will begin by the end of the year.

Significant Accomplishments

During the past 2 1/2 years (1) We have proven that there is a significant source of copper to the surface waters of the ocean, with the most striking signals to date being encountered in the Gulf of Panama, Western North Atlantic shelf waters, Mediterranean, and Northern Gulf of Mexico. This work has involved generation of a data base larger than the total literature of reliable trace element analyses. (2) We have discovered that nickel and cadmium also show significant enrichments in the surface waters of the Mediterranean relative to similar nutrient-depleted water in the open ocean. (3) We have developed a simple and reliable towed

system for collecting trace element samples underway. (4) We have designed and partially tested a new deep water sampler for contamination-free trace element profiling. (5) We have begun development of a model for processes controlling trace element concentrations in the surface waters of the ocean; this model and the data base mentioned above will appear in a forthcoming major paper in the Journal of Geophysical Research.

REDUCED GAS AND SUSPENDED PARTICULATE STUDIES IN THE OCEAN

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Long-Range Scientific Objectives

Our objective is to understand the processes controlling the geochemistry of reduced gases (CH_4 , N_2O , H_2 , CO , $\text{C}_2\text{-C}_4$ hydrocarbons, NH_3) in ocean waters and sediments.

Project Objectives

1. To identify and quantify those oceanic processes, whether biological (via bacteria, phytoplankton, or zooplankton) or physical (accumulation of particulates on oceanic thermoclines) that control near surface distributions of reduced gases.
2. To understand the processes controlling gas production, consumption, and mobility throughout the sedimentary column.
3. To develop a liquid chromatographic-total scanning fluorescence technique as a method for studying suspended and dissolved (plant pigments and petroleum products) species in the ocean.

Current Status of the Project

1. A major portion of our reduced gas effort is a cooperative effort between Texas A&M and NORDA (Code 334). Our laboratory has field responsibilities for reduced gas measurements and NORDA for most of the suspended particulate, biological, and hydrographic parameters, although specific analytical responsibilities for a field exercise vary depending upon the work load of each laboratory. In addition to our formal involvement with NORDA, we are also working with the Environmental Chemistry Section of NRL on reduced gas research. We have participated in both a NORDA and NRL cruise each of the last three years.
2. The major emphasis of our program during 1981 is an evaluation and publication of the data collected during a series of 4 to 5 major cruises in 1979 and 1980. The effort this year will result in three or four papers on reduced gases in the upper water column. Our only major field effort in 1981 was a South Atlantic cruise with NRL aboard the USNS Hayes.
3. Another major area of reduced gas research we are pursuing is the study of gas in marine sediments. These studies have dealt initially with gas hydrates in marine sediments. An initial study was undertaken on Leg 76 of the DV Glomar Challenger as part of the DSDP-IPOD program. This cruise accomplished four pressurized core barrel (PCB) samplings of hydrate gas at *in situ* pressures. Pressure release experiments were conducted in the field and samples were obtained for laboratory molecular and stable isotope studies. The hydrate studies have resulted in several presentations and will result in a minimum of four publications. We will participate in another hydrate research leg of the DSDP-IPOD program in the Middle-Americas Trench in January 1982.

4. We have also undertaken initial laboratory experiments to study reduced gas production by phytoplankton. These studies are in cooperation with the hydrogen program of Dr. Schink at Texas A&M University.
5. A total fluorescence spectra technique has been developed in which high resolution three-dimensional arrays of excitation, emission, and peak intensities are obtained using a scanning fluorometer interfaced to a microcomputer with graphics. This system is also being interfaced to a liquid chromatograph for fluorescent studies of dissolved and particulate organic species.

Significant Progress

1. Reduced gas anomalies have been observed in the Eastern Tropical North Pacific, Mediterranean Sea, South Atlantic, North Atlantic, Gulf of Mexico, and Caribbean Sea and appear to be fairly ubiquitous in the upper water column of the ocean. Methane maxima are generally associated with the upper part of the thermocline and reach levels of roughly twice surface equilibrium concentrations for a wide variety of open ocean areas. In coastal areas of the Gulf of Mexico, subsurface methane maxima have been observed that are several hundred times surface concentrations. These maxima in most cases appear to be supported by *in situ* biological production either from bacteria associated with reducing microenvironments or guts of zooplankton, or from phytoplankton which also often show maxima associated with the thermocline. Deep methane maxima have been observed in the northwestern Caribbean Sea probably as a result of seepage along the Jamaican Ridge system. Nitrous oxide also shows surface maxima in the upper few hundred meters of the water column, not associated with oxygen depletion as observed in deep water.
2. Our DSDP gas hydrate studies aboard the DV Glomar Challenger accomplished the first successful sampling of gas hydrates in deep ocean sediments using a pressurized core barrel. These observations were the first confirmation that the bottom simulating reflector (BSR) observed in the Blake-Bahama Outer Ridge System is actually associated with methane hydrate containing sediments. Pressure release curves obtained from the PCB were comprised of up to three distinct intervals: initial hydrostatic pressure loss; gas hydrate decomposition; and sediment degassing. Gas expansion due to decomposing gas hydrates was about 13:1. Ca. 56% of the volume was attributable to releasable gas present in a mole ratio of 50:1 (water:gas) corrected for pore fluids. The sediments remaining after degassing were ca. 93% water by weight. Hydrocarbons larger than $i\text{-C}_4$ were not present in the hydrate lattice.
3. Our laboratory has also developed a computer based total scanning fluorometer. This system has the advantage over video fluorometers currently in use to obtain three-dimensional data in that a five fold increase in resolution is obtained, instrumentation costs are roughly half, and our instrumentation/computer/graphics package has already operated successfully at sea.

CAUSES OF CHEMICAL VARIABILITY IN THE OXYGEN-DEFICIENT
WATERS OF THE OPEN OCEAN

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Long-Range Scientific Objectives

Our long-range goal is an improved understanding of the major open ocean oxygen deficient zones found in the Eastern Tropical Pacific and in the Arabian Sea. To do this, we employ a multidisciplinary approach that includes improved time-series data arising from our instrument development efforts and an extensive suite of chemical, physical, biological and biochemical data.

Project Objectives

To improve our understanding of the processes that contribute to the formation and maintenance of the secondary nitrite and particle maxima found in open-ocean oxygen deficient zones, to better estimate denitrification and nitrification rates within these strata, and to develop instrumentation that will provide improved chemical time-series data from these zones and from other environments.

Current Status of the Project

During the current year, we have been perfecting and testing a moored water sampler, and analyzing data from past experiments and from a data base that we have compiled for the Eastern Tropical South Pacific. We also participated in a cruise to the Eastern Tropical North Pacific, and we are now analyzing the results from this experiment. Two papers dealing with aspects of this project are being prepared and it is anticipated that two more will be started before the end of the current contract year.

Significant Progress

During the 1979-1981 period, ten papers dealing with aspects of our ONR research have been published or are in press. The most significant findings of these completed studies and of our ongoing research may be summarized as follows:

1. Conditions in the main secondary nitrite maximum (MSNM), a sub-surface feature found off western South America between 10-25°S changed markedly in recent years, and it appears that the increased nitrite concentrations are associated with increased denitrification rates.
2. The recent denitrification rate in the oxygen deficient waters off western South America is about 2.5×10^{13} g N yr⁻¹ making this region one of the most important sites for marine denitrification.

3. Most of the denitrification (about 2×10^{13} g N yr⁻¹) occurs in the coastal portion of the MSNM. Small and shallow maxima, a deep maximum found near 5°S, oxygen deficient bottom layers found over the shelf and upper slope, and the nearshore sediments themselves, make contributions of 0.2, 0.1, 0.1 and 0.2×10^{13} g N yr⁻¹, respectively.
4. Particle maxima have been found in the MSNM, the deep maximum near 5°S, and in the secondary nitrite maximum found in the Eastern Tropical North Pacific, and some of the mechanisms that may lead to their formation have been described.
5. We have shown that the dominant nutrient variability scales in the upwelling ecosystems that overlie some of the oxygen deficient waters that we study, may differ from region to region, and we have discussed some of the physical processes that may cause them to differ.
6. We have shown a correlation between coastal trapped waves and anomalous nitrite distributions off Peru.
7. We have described the effects that a subsurface nutrient "front" off Peru has on the nutrient concentrations of the waters that upwell at 15°S.
8. We have brought the design of our moored water sampler to a stage where we can begin long-term reliability tests and begin to use it for shorter term experiments. In addition, we have published a short description of the sampler.
9. We have provided a description of the nutrient fields off Somalia during the S.W. Monsoon of 1979, and shown that two distinct upwelling centers existed during our observational period, one near 5°N and the other in the "traditional" location near the "horn of Africa" (~ 10°N).
10. We have described the evolution of the nutrient regime in the poleward undercurrent off Peru as it travels from 10 to 16°S.

OCEANIC DISTRIBUTION OF
TRACE METALS

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Long-Range Scientific Objectives

To establish the distributions of the trace metals and other minor species in the ocean and to establish the mechanisms controlling these distributions.

Project Objectives

To develop accurate, precise and rapid techniques for trace metal analyses which can be used at sea. Methods to be based on the electron-capture gas chromatography of volatile organic chelates of the metals.

Current Status of the Project

Since the initiation of the project on 1 January 1981, efforts have been directed at the characterization of synthetic chelates of various metals (Cu, Ni, Zn, Cr, Al). Promising results have been obtained for all these species with the exception of zinc. The effort is now being directed at formation of the complexes in sea water and their extraction into an organic phase.

A small field program has been completed on the differential uptake of the two oxidation states of Selenium (SeIV and SeVI) by marine phytoplankton. Using existing g.c. methods, waters from a coastal upwelling area were sampled during bloom conditions in conjunction with biological monitoring of chlorophyll, particulate chemistry etc. It was found that the phytoplankton exert an almost exclusive preference for the reduced species SeIV.

Significant Progress

The results of the selenium work have been submitted as a short paper to NATURE.

A publication of results from the previously funded oceanic hydrothermal program will shortly appear in EARTH AND PLANETARY SCIENCE LETTERS.

SHIPBOARD TRACE METAL ANALYSES

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Long-Range Scientific Objectives

Our ability to study trace metal chemistry in the ocean has been limited by the necessity to store samples and return them to a shore-based laboratory for analysis. Shipboard analyses would permit an interaction between the analytical results and the sampling strategy and they would make it possible to conduct experiments at sea on fresh samples which is important when addressing chemical speciation questions. We are developing the capability to perform trace metal measurements underway.

Project Objective

Our objective is to establish the ability to perform atomic absorption spectroscopy (AAS) measurements at sea. The two principal analytical techniques with adequate sensitivity for oceanic trace metal measurements are anodic stripping voltammetry (ASV) and graphite furnace AAS. We have used ASV at sea in our earlier work, but it is limited to copper, cadmium, zinc, and lead measurements in seawater. In addition, the electrochemical measurements depend on the speciation of the metals in a manner which is not well understood. We have chosen AAS for this work because it is applicable to a wide range of chemical elements and the interpretation of the analytical results is fairly straightforward. The project consists of being able to operate an AAS instrument onboard a research vessel and of providing rapid, nearly real-time measurements of selected trace metals while underway.

Current Status of the Project

This project has consisted of four major tasks in its first full year of work. We examined a number of commercially available AAS instruments and selected the Perkin-Elmer 5000 with a Model 500 Graphite Furnace as best suited for this application. To our knowledge no one has successfully obtained reliable trace metal measurements by AAS while at sea. There are several potential problems that we have considered in pursuing this work including metal contamination aboard ship, the need for temperature and humidity control, the effects of gravitational accelerations due to ship motion, and vibration from the ship's machinery. We have constructed an 8' x 16' portable trace metal laboratory to provide a suitable environment for the AAS instrument. Our first test cruise is scheduled aboard RV ENDEAVOR during September 1981. The AAS measurements require preconcentration of the metals prior to analysis. In a shore based laboratory we used the cobalt-APDC coprecipitation technique with high speed centrifugation. This method is time consuming and not well suited for use at sea. We have developed a new preconcentration system which can be automated and provides samples more rapidly than the coprecipitation. We have used a closed flow system constructed of Teflon to add reagents to a sample and to extract the metals on a substrate. The concentrated metals are then eluted from the substrate and analyzed by AAS. The fourth aspect of this work is a sampling system. Eventually we anticipate using a non-contaminating submersible pumping system for nearly continuous underway measurements, but

for the development of our analytical system we have obtained a 24 position 5 liter bottle rosette sampler which is mated to a CTD-O₂ sensor system. The samplers are Teflon lined General Oceanics GoFlo bottles.

Significant Progress

After this first year's work we have completed the design and construction of the shipboard trace metal laboratory, we have obtained extensive experience with the AAS instrument, we have developed several prototype automated preconcentration systems, and we have set up a discrete chemical sampling system.

Future Plans

During the coming year we plan to refine the shipboard analysis system based on our experience during the September 1981 field work. We are scheduled to perform additional shipboard measurements with the system in 1982 on two of the Warm Core Rings cruises. This work will provide an opportunity for two three-week cruises during which there will be extensive chemical, biological, and physical oceanographic measurements in an oceanic frontal environment where we can expect substantial chemical gradients and fluxes.

IMPROVED DETECTION OF OXYGEN CONSUMPTION,
NITRATE REDUCTION, AND CO₂ EVOLUTION IN THE DEEP-SEA

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Long-Range Scientific Objectives

To develop sensitive, "real-time" techniques for measuring the rates of oxygen consumption, nitrate reduction and CO₂ evolution in the deep-ocean.

Project Objectives

1. To investigate the mechanisms by which oxygen consumption and nitrate reduction are regulated. We suspect that regulation is accomplished by the relative levels of adenosine di- and triphosphate in the marine microorganisms that are responsible for the utilization of O₂ and NO₃ in the sea. Our investigation will be focused on this theoretical mechanism.
2. To explore fluorometric and electrochemical methods of measuring enzyme activity in marine microbes.
3. To use our methodology to investigate rates of oxygen utilization, nitrate reduction and CO₂ evolution in the deep and intermediate waters of the Indian Ocean, the Pacific Ocean and the Mediterranean Sea.

Current Status of the Project

1. We have measured the potential rates of oxygen consumption, nitrate reduction and CO₂ evolution in the deep-waters under the Peru Current and in the northeastern tropical Pacific. Now we are in the process of calculating in situ rates from this data and publishing the results; three papers have already been published (Garfield et al., 1979, Codispoti and Packard, 1980, and Packard and Garfield, 1980) and three are in the manuscript preparation phase. One will describe the rate processes in the trench waters under the Peru Current; the other two will describe enhanced oxidation rates associated with particle maxima in and around the oxygen minimum zones off the Mexican and Peruvian coasts.
2. We have developed a fluorometric enzyme assay of respiratory electron transport activity and are in the process of refining it to the point where it is more sensitive and effective than the existing spectrophotometric assay.
3. We are staging, for an expedition on the French oceanographic vessel, *Jean Charcot*, to measure respiratory electron transport activity in the bottom waters of the western Mediterranean Sea. From these

measurements, we will calculate oxygen consumption, CO₂ evolution and nitrate and phosphate remineralization for the deep water as it moves from its source.

4. We are writing a review paper to be included in a book entitled "Coastal Upwelling: Its Sediment Record" that will summarize the results of the NATO Advanced Research Institute held in Algaré, Portugal on 1-4 Sept., 1981. Our review will summarize our results in using enzyme analysis in studying deep-sea oxidation processes.

Significant Progress

1. Real-time detection of oxygen consumption, CO₂ evolution and nitrite reduction can be accomplished by enzyme analysis of water samples.
2. Deep-sea oxygen consumption proceeds at a rate of about 4 $\mu\text{l O}_2 \text{ yr}^{-1} \text{ l}^{-1}$ (at 2000 m). This is a thousand-fold less than the rate in the surface waters.
3. Nitrate reduction in anoxic waters proceeds at about 0.09 $\mu\text{g-at day}^{-1} \text{ l}^{-1}$. On a global scale, this can cause a loss of $6 \cdot 10^{13}$ g nitrogen per year from the oceanic water column.
4. The intensity of the oxygen minimum zone is dependent on the metabolic activity of the in situ microbes which is, in turn, dependent on the magnitude and stability of the surface productivity.
5. These results have been presented orally at meetings of the American Society of Limnology and Oceanography (Jan. 1979, Jan. 1980), a meeting of the British Challenger Society (Jan. 1981), at a NATO Research Institute (Sept. 1981), and at a special seminar at the University of Maryland.
6. These and other results have been published in the following articles:

Codispoti and Packard (1980); Garfield et al. (1979); Packard and Garfield (1981); Packard and Williams (1981) and Setchell (1981).

EXAMINATION OF RADIUM-COPPER COVARIANCE IN THE SURFACE OCEAN AS EVIDENCE FOR DIFFUSIVE INPUT OF COPPER FROM SEDIMENTS

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Long Range Scientific Objectives

To identify and understand mechanisms responsible for supplying and controlling the distribution of dissolved copper in the surface ocean.

Project Objectives

To test for evidence of diffusive input of dissolved copper from near-shore sediments to surface waters of the ocean by determining if copper and radium (^{228}Ra , ^{226}Ra) covary in areas of the surface ocean contiguous with the continental shelf.

Current Status of the Project

A cooperative effort has been established between this lab performing the radium sampling and analyses and Dr. E. A. Boyle at Massachusetts Institute of Technology, who carries out the joint copper sampling and analyses under a separate existing ONR-funded project. Three simultaneous sets of surface radium and copper samples have been collected. The first was obtained in the Mediterranean Sea between 21°E and 8°W . Another set was obtained in both near-shore and off-shore waters of the Eastern Pacific between San Diego, CA and Acapulco, MX. The third set of joint samples was obtained in the eastern Gulf of Mexico on a transect through the Loop Current into the northeastern shelf and coastal zone. Processing and analyses of these sample sets have been initiated and are in various stages of completion.

Significant Progress

1. The analyses of copper and ^{226}Ra samples from the Mediterranean Sea have been completed. The concentrations of both parameters are greater in the more saline Mediterranean Surface Water than in the inflowing North Atlantic Surface Water. The increases in concentrations clearly exceed that which could be attributed to evaporative processes producing Mediterranean Surface Water. When Cu and ^{226}Ra are plotted together, a linear regression fits the data with a coefficient of variation (r^2) of +0.72. These comparisons provide the first direct evidence for radium-copper covariances in the ocean. We hope to confirm these observations with the ^{228}Ra measurements now in progress.
2. The complete Mediterranean data set, including ^{228}Ra , and at least one other complete data set will be submitted for presentation at the Spring Meeting of the American Geophysical Union.
3. One or more papers derived from the three existing sample sets will be submitted for publication during 1982.

CHEMICAL VARIABILITY IN THE UPPER OCEAN

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Long-Range Scientific Objectives

To understand the processes responsible for chemical variability in the upper layers of the ocean.

Project Objectives

To utilize close-spaced, continuous underway measurements of a number of chemical and physical parameters in order to better understand mixed layer processes. In particular to determine the relationships between dissolved H₂ and CO and other properties of the upper ocean.

Current Status

1. We now have operational a towed pumping system that can collect water (at 6 l/min) from depths to 135 m, while the ship is underway at 10 knots.
2. Analytical systems have been assembled to determine H₂, CO, in vivo fluorescence, nutrients (silicate, nitrate, nitrite, phosphate), salinity and temperature on a pumped water stream continuously while underway and to log the data in a computerized data management system.
3. Recent field work (1979-81) is under analysis. Laboratory and meso-scale tank (i.e. MERL) studies are in progress.

Significant Progress

The following conclusions are contained in papers now in press or under review for publication in the open literature. Papers have been or are being submitted to the AGU-ASLO meeting Feb. 1982, and JOA meeting in Halifax:

1. Hydrogen, carbon monoxide, and chlorophyll-a were studied in CEPEX containers where advection and vertical diffusion was nil. A chlorophyll bloom was followed by an increase in H₂ within the bags, but at different depths than the chlorophyll. The H₂ increase is attributed to grazing animals producing H₂ in their guts; however, it is possible that nitrogen-fixing phytoplankton were the source. Carbon monoxide inventory was related to light intensity and was far greater in the biologically-rich CEPEX bags than the CO inventory in Central Pacific waters. However, the vertical distribution of CO in the bags was very different from the vertical distribution of available light.

2. In open ocean waters the CO concentration correlates well with sine T, where T represents hours from noon. Four different zones with differing water characteristics gave distinctly different slopes and intercepts for the CO vs. sine T regression. Waters with greatest average in vivo fluorescence had greatest average CO and greatest amplitude in the daily CO variations. Fluorescence and CO daily cycles were similarly related in the other waters with least fluorescence found in waters with least average CO and least amplitude of daily cycles.
3. A daily cycle of H₂ concentration is apparent in tank studies at CEPEX and at MERL, but no significant daily effect has yet been established in open sea surface measurements.
4. Hydrogen concentrations were strongly correlated with in vivo fluorescence values at the surface in warm waters reaching out 100 km from the Southern California coast. Similar correlation was absent in bands of cooler water out to 550 km.
5. H₂ and CO concentrations in sea-surface air of the Central Pacific during July-Sept. 1978 were found to be lower than normally reported. CO values rose markedly to the north of the Intertropical Convergence, but H₂ values did not. Higher values to the north are generally attributed to anthropogenic sources, but such sources should cause both gases to increase. This suggests that these gases have shorter residence times in the atmosphere than generally believed.
6. Signal theory has been applied to continuous underway sampling systems to derive the gain function of a hose/dead-volume combination. Resolution of concentration variations is degraded in proportion to the length of the hose and the speed of the ship. Resolution can be improved by increasing the pump pressure at the bottom end of the hose, but only as the one-half power of the pressure.

Plans for the Future

1. We plan to deploy the towed pumping system to study shelf-slope-warm core ring transitions in the Summer of 1982, immediately following the Warm Core Rings program three-ship cruise.
2. We plan to make continuous underway measurements across the Peru upwelling region in conjunction with Dana Kester in Spring 1983. Other collaborators will be sought.

THE ROLE OF CYANOBACTERIA IN THE MARINE HYDROGEN CYCLE

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Long-Range Scientific Objectives

The long range goal of this project is to determine which biological processes are responsible for controlling the concentrations of hydrogen and other reduced gases in the oceans.

Project Objectives

To investigate the relationship between hydrogen concentrations in the ocean and biological activity. As part of this effort we have begun an evaluation of the importance of a specific organism, *Oscillatoria thiebautii*, as a source of hydrogen for the oceanic mixed layer.

Current Status of the Project

The project objectives were accomplished as follows:

1. A hydrogen analyzer, based on the one developed by Herr and Barger of the Naval Research Laboratory, was constructed. Instrumental sensitivity is sufficient to detect about 1 nM of hydrogen.
2. Hydrogen data were collected throughout the Mediterranean and Gulf of Cadiz in August 1980 on cruise 1309-80 of the USNS Bartlett. Vertical profiles were obtained in most of the major basins of the Mediterranean. Horizontal variability studies were also undertaken to permit an evaluation of the patchiness present in mixed layer hydrogen distributions.
3. A field experiment at the West Indies Laboratory in St. Croix was also carried out. Samples of *Oscillatoria thiebautii* were collected, and short term (1-3 hour) assays for acetylene reduction (nitrogen fixation) and hydrogen production were made. Water samples, obtained from positions where *O. thiebautii* was collected, were also analyzed.

Significant Progress

Important results were obtained in two major areas during the current year. Firstly, our hydrogen analyses in the Mediterranean demonstrated that, as in other oceans, surface waters are substantially supersaturated in hydrogen relative to equilibrium with the atmosphere and the deep waters are at or slightly below equilibrium. Horizontal drift experiments in which samples were taken every 100 m or so at depths of 20 and 100 m suggest that substantial patchiness (presumably due to biological activity) exists over quite short distances (few hundred meters) especially at 20 m. These observations confirm our postulate that excess hydrogen is biologically produced.

In contrast to the hydrogen distribution observed throughout much of the Mediterranean, we found anomalous hydrogen concentrations near the Strait of Gibraltar in the Alboran Sea. At depths ranging from 100 to 400 m or 1000 m (two stations), large hydrogen maxima were observed. Because of the presence of these maxima at depths just below the strong density gradient between outgoing Mediterranean water and inflowing North Atlantic water, we hypothesize that hydrogen production may occur within particles held up on the density interface. A paper entitled "Distribution and variability of hydrogen in the Mediterranean" by MI Scranton, MM Jones and FL Herr has been submitted to Limnology and Oceanography.

The second major set of data obtained during the current contract year is the result of our field work in St. Croix. Using short term incubations of *Oscillatoria thiebautii* collected off the north shore of St. Croix, we have conclusively shown that these organisms produce hydrogen in significant amounts. Based on hydrogen production to nitrogen fixation ratios determined in these experiments of about 0.07 $\mu\text{moles H}_2$ produced per $\mu\text{mole N}_2$ fixed, we feel that these organisms, when present, probably represent an important mixed layer source of hydrogen. A manuscript tentatively entitled "Oceanic hydrogen production by a pelagic marine cyanobacterium, *Oscillatoria thiebautii*" by MI Scranton is in preparation.

SATELLITE AND SYNOPTIC STUDIES OF CHEMICAL FRONTS IN THE CALIFORNIA CURRENT AND COASTAL UPWELLING ZONE

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Long-Range Scientific Objectives

The long-range objectives of this research are to develop bio-chemical models which will lead to an understanding of the role of chemical fronts in the ocean; their effect in production and distribution of microplankton and the biogeochemical transport of non-conservative elements; and to couple bio-chemical models to predictive models based on satellite remote sensing for the purpose of forecasting important bio-chemical, acoustic and optical properties.

Project Objectives

Specific objectives of this project are to combine satellite and shipboard observations to detect and describe oceanic frontal systems which are formed by the interaction of the California Current and coastal upwelling zone; to measure bio-chemical parameters which will lead to an understanding of the relationships between satellite detected thermal patterns and nutrient structure of the sea surface, between nutrient fronts and *in situ* and satellite detected distribution of microplankton, between frontal nutrient flux and production of total biomass, between different frontal systems as a function of bio-chemical age, structure (e.g., cyclonic or anticyclonic) and seasonal insolation and variation of water masses; to begin the development of predictive models by converting satellite images to nutrient and biomass maps of the sea surface; to extrapolate the sea surface signature to contiguous subsurface distribution of these properties and to formulate and test a unifying bio-chemical model.

Current Status of the Project

During the current year we have seen the successful completion of important objectives including (1) the conversion of satellite infrared images to nutrient maps, (2) the remarkable agreement between *in situ* inferred distribution of chlorophyll biomass in thermonutrient fronts and the distribution of chlorophyll inferred from the Nimbus-7 Coastal Zone Color Scanner (CZCS), (3) the capability of analyzing production over large frontal regions by using guanosine triphosphate (GTP) for determining microplankton growth rates, and (4) the completion and successful performance of the towed oceanwater sampling system (TOSS).

Significant Progress

By coordinating satellite and shipboard observations we have demonstrated the existence of cyclonic upwelling systems off Pt. Sur, California. As viewed from space these features appear to form by the intrusion of warm California Current waters into the coastal upwelling zones where they interact with upwelling water to form a cyclonic circulation. Our conceptual model of the 'cyclonic upwelling system' appeared on the cover of EOS Transactions of the American Geophysical Union, 62(36), 8 Sept. 1981.

Interaction between cold coastal waters and warm oceanic waters produce cyclonic and anticyclonic frontal systems and seaward surface flows of cold water with a large scale redistribution of nutrients. This is a significant factor in determining primary production and distribution of biomass along the coastal boundary and in the California Current. Off Pt. Sur recurrent injections of cold, nutrient-rich waters upwell from depths of tens to hundreds of meters and frequently produce a cyclonic structure in the surface thermal and nutrient field. These systems have been observed to spin up into tightly curled cyclonic features in less than 48 hours and persist for 10 days or more. Some move poleward into Monterey Bay, others dissipate in place. Ships are too slow to study the synopticity of these large (~100 km in diameter) and rapidly changing systems. However because of excellent inverse correlations between nutrients and temperature, we have been able to convert satellite IR thermal images to sea surface nutrient maps. These "maps" demonstrate the mesoscale location of sharp thermal and nutrient fronts which we have discovered in situ. Analysis of in situ data have shown that microplankton are concentrated in these thermochemical gradients and particularly on the equatorward side of the cyclonic cold water-warm water boundary, showing a unique relationship between chemical structure and biological distributions in the eastern boundary region. Convincing support for this observation was obtained by the remarkable agreement between in situ data and color imagery from the Nimbus-7 coastal zone color scanner. Because these features persist and phytoplankton blooms are located in the sharp nutrient gradients, we have suggested that they may act as 'natural chemostats' in which the specific growth rate is equal to the dilution rate and total biomass is proportional to the concentration of the nutrient supply. Our new GTP measurements show a relationship between fronts and high absolute production. GTP/ATP ratios show high specific growth rates which are about one-third those of hydrothermal vents. These measurements are a step toward evaluation of the chemostat concept. In the future with the aid of TOSS, greater emphasis will be placed on seasonal insolation and 3-dimensional seasonal water mass variability which appears to be the most significant factor in determining the initial conditions of frontal systems.

Twelve publications, three of which are full papers, are listed in the bibliography under Traganza or Traganza et al. Eight master's theses have been printed by the Naval Postgraduate School and are referenced in the above. Three invited talks have been given to international audiences, viz., at the I.D.O.E. International Symposium on Coastal Upwelling, the Gordon Research Conference on Chemical Oceanography and the NATO Advanced Research Conference on Coastal Upwelling: its Sedimentary Record. This project has attracted the attention of numerous scientists. Some have initiated studies off Pt. Sur, including e.g. dynamic studies by Dr. C.N.K. Mooers and L.C. Breaker (a Ph.D. student) of NPS and Dr. R. Smith of OSU, CZCS satellite studies by Dr. J. Mueller of NPS, and acoustic studies by Dr. P. Scully-Powers and D. Browning of NUSC.

Dr. Neil Andersen of NSF has used this work as an example of the future potential role of satellites in chemical oceanography. Dr. R. Stevenson and Mr. Ben Cagle of ONR have extrapolated our findings to an area of strategic Naval importance and suggested that our region should be used as a model representative of strategic areas of the ocean for oceanographic studies in an interactive mode with Naval operations.

Future Plans

Dr. Traganza will be on sabbatical leave at NRL Washington D.C. (1 Oct 1981 - 31 Dec 1981) and SIO, La Jolla Ca. (1 Jan 1982 - 30 Sept. 1982) where he will continue this research. Emphasis will be on nucleotide chemistry of microplanktonic production. A two week cruise on the R/V ACANIA is planned with the SIO marine food chains group in May 1982. Points of contact are Dr. Conrad Cheek, NRL and Dr. Richard Eppeley, SIO.

REAL-TIME MARINE CHEMISTRY

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Long-Range Scientific Objectives

To be able to monitor and predict the chemical properties of the surface ocean in real time.

Project Objectives

To develop electro analytical methods and instrumentation for real-time ocean monitoring. To determine spatial and temporal variability of trace metals, pH and other variables in the surface ocean.

Current Status of the Project

1. A microprocessor controlled instrument for the direct voltammetric analysis of Zn, Cd, Cu and Pb has been developed and constructed. The instrument carries out measurements directly and semicontinuously on a stream of seawater. By means of specially written software, the instrument can calculate charge transfers equivalent to 0.01 ppb of Cu.
2. In the past year we have attempted to make underway Cu measurements on the NOSC-sponsored VARIFRONT II expedition to the eastern tropical Pacific Ocean. A trace-metal free pumping system was mounted below the bow of the R/V DESTEIGUER and water was pumped to instruments in the ship's laboratory. Underway measurements of surface chlorophyll, pH, bioluminescence, temperature, NO_3^- and Cu were made from San Diego to Acapulco. Cu concentrations were found to group around two values: Baja California coastal waters averaged 0.20 ± 0.16 ppb ($n=31$) while values from an oligotrophic area approximately 200 miles off the coast averaged 0.08 ± 0.05 ppb ($n=24$).
3. An intercalibration experiment carried out in conjunction with E. A. Boyle of MIT indicated that Cu values measured with the automated instrument average out to values similar to those obtained by APDC co-precipitation. The level of precision is, however, much poorer.
4. Other projects currently underway include a continuing study of the properties of the Cu(II) ion selective electrode in seawater, a differential measurement of the temperature dependence of the pH of seawater, a study of voltammetric peak currents as indicators of trace metal speciation, an effort to calculate primary productivity from pH measurements made in the equatorial upwelling zone and an improved electrochemical technique for the measurement of NO_3^- in seawater.

Significant Progress

A device for the shipboard measurement Cu, Zn, Pb and Cd in ocean waters has been constructed. This instrument has been used in estuarine, coastal and open ocean waters. It was shown that the Cu and Zn concentration in San Diego Bay followed tidal cycles. In the Peruvian upwelling system Cu was found to decrease with increasing primary productivity. It was demonstrated that Cu concentrations in seawater (as measured electrometrically) decreased with increasing pH of seawater. A speciation model for Cu was suggested to explain this behavior.

Cells were devised for the precise measurement of the pH of seawater using either discrete samples or continuous streams. It was shown that in the Peruvian upwelling system, surface and near-surface pH were controlled almost entirely by biological processes. A model which estimates primary production from changes in pH is being developed.

It was suggested that the Cu(II) specific electrode can measure Cu(II) in seawater in flowing streams through a redox process. It was demonstrated that the electrode potential responds to the oxygen concentration in solution. Other studies are underway to better understand the mechanisms of its operation.

The first theoretical study of stripping polarography was undertaken with the purpose of extending the sensitivity of anodic stripping voltammetry to the direct study of speciation. A computer-controlled polarograph was constructed which automatically produced stripping-polarograms of Cu, Cd and Zn in seawater. The original work was expanded to include complex ions in seawater.

STABILITY OF MATERIALS AND COMPOUNDS IN THE OCEAN

THE ORGANIC CHEMISTRY OF SEA WATER: COMBINED AMINO ACIDS,
 α -KETO ACIDS, AND DICARBOXYLIC ACIDS

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Long-Range Scientific Objectives

The principle long-range goal of this research is to gain a better understanding of the cycle and characteristics of low molecular organic compounds in sea water.

Project Objectives

Our investigations have focused on combined amino acids, α -keto acids and dicarboxylic acids. These compounds are involved in several fundamental biochemical pathways, and thus by studying the distribution of these compounds in the oceans we should be able to enhance our knowledge of the cycle and fate of important biologically derived organic compounds in the sea.

Current Status of the Project

This laboratory has carried out extensive investigations of the concentration and enantiomeric composition of combined amino acids in various oceanic waters. The concentration and composition of the combined amino acid material in surface waters has been found to differ substantially from that in deep waters. In the deep ocean, alanine accounts for nearly 70% of the combined amino acids, while in surface waters it accounts for only ~30%. Moreover, the alanine in deep waters has also been found to be essentially racemic, i.e., D/L alanine \approx 1. These observations can be explained by invoking a diagenetic reaction in which serine undergoes a dehydration reaction, yielding racemic alanine. This mechanism also predicts the presence of α -amino-n-butyric acid (ABA) in the combined amino acids in the deep ocean; ABA is produced from threonine via a dehydration reaction analogous to that for serine. ABA was detected in deep oceanic waters and it was found to be racemic, in accordance with the proposed threonine pathway. This is the first time evidence has been obtained which suggests that chemical diagenesis of organic material occurs in the oceans.

Filtration experiments carried out on various sea water samples indicate that the bulk of the combined amino acids are associated with a dissolved component, and not colloids or particulates. In addition, combined amino acids are not part of the XAD-2 humic-acid like material which is present in sea water.

The results of the investigation of the presence of dicarboxylic and α -keto acids in oceanic waters indicate that measureable quantities of oxalic, glyoxalic and pyruvic acids are present in near shore surface waters, while very low and undetectable quantities of these compounds are found in both surface and deep water open ocean samples.

Significant Accomplishments

Our investigations indicate that the bulk of the combined amino acids in sea water are associated with some dissolved component, and that the serine and threonine present in this component undergo irreversible dehydration, producing racemic alanine and ABA, respectively. These results thus provide some of the first direct evidence that chemical diagenesis of dissolved organic material takes place in the sea. Chemical diagenetic reactions such as dehydration could greatly affect the biological usability of the dissolved organic material in the oceans. It is conceivable that chemical diagenetic reactions, such as serine and threonine dehydration, may partially account for observations that the total dissolved organic carbon in sea water tends to be fairly biologically inert, and has an average lifetime in the oceans of many thousands of years.

Our work on α -keto and dicarboxylic acids, which are primary metabolites and thus important in biological energy cycling, indicates that these compounds are present in the open oceans in only very low concentrations. These compounds are apparently highly labile, and are rapidly consumed and utilized by marine organisms.

A STUDY ON THE TRANSFORMATIONS AND FATE OF ORGANOTIN MATERIALS AND COMPOUNDS IN MARINE ENVIRONMENTS

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Long-Range Scientific Objectives

Our long-range goals are to advance the underlying aquatic organometallic chemistry of tin with concomitant development of new ultratrace measurement techniques that permit assessment of molecular forms, lifetimes, and fate of both naturally-occurring and man-made organotin materials in marine environments. Such an interdisciplinary approach makes available both tin- and ligand-specific predictive models for speciation of organotin biocides in the presence of microorganisms involved as precursors in marine fouling, thereby leading to effective design of tailored controlled release organotin polymers with reduced environmental impacts.

Project Objectives

To develop molecular speciation techniques for characterization of model trace organotin compounds in saline environments; to investigate biological and nonbiological transformations of selected organotins which may occur in marine environments; and to correlate the effects of selected organotin structures with respect to inhibition of aquatic microorganisms responsible for fouling.

Current Status of the Project

During the current year, at ONR's request, we reduced efforts on characterization of organotin polymer (OMP) candidates for controlled release antifoulants and concentrated on applying newly developed gas and liquid chromatographic speciation methods to questions of naturally-occurring methyltins and biodegradation of anthropogenic butyltins found in the marine environment. These efforts include: a) demonstration of a novel purge and trap sampling system coupled to a GC fitted with a tin-selective flame photometric detector (FPD); b) discovery of both volatile tetramethyltin (TMT) and methyltin hydrides along with involatile ionic methyl- and butyltin species at ng/L concentrations in estuarine waters; c) proving misassignment of Sn(IV) as only aquatic inorganic tin species based on conventional hydride analyses since Sn(II) competitively also forms stannane; d) quantitation of cellular (membrane) bioaccumulation of biocidal tributyltin cation by 13 tin-resistant Chesapeake Bay bacteria with little reduction of metabolic activity or degradation of tin-carbon bonds; e) determination of a linear correlation between the Hansch-Leo solvophobic parameter (a direct measure of lipophilicity or membrane transmission) for both alkyl and aryl groups and the thermodynamic free energy term experimentally obtained from the logarithm of organotin HPLC retention times (capacity factor); f) a comparable additive correlation between respective tin-119 NMR chemical shifts and HPLC retention times for series of alkylaryl tin compounds; g) development of a HPLC method for measuring lifetimes of refractory organotin hydrides in aqueous media, pseudo first-order hydrolysis of model tributyltin hydride gave $t_{1/2} = 6.2$ h; h) use of a HPLC delivery system for GC-FPD measurements of organotin aqueous solubility and estimations of their air-water partition coefficients, including TMT; i) analysis of

field data and laboratory model experiments designed to assess by purge and trap GC-FPD possible biodegradation of tributyltin compounds in Chesapeake Bay sediments and the nature of organotin species release to air above surface waters. Results from d, e, f, h, i, are in preparation for publication.

Significant Accomplishments

During the past three years we have developed and demonstrated two new independent methods for ultratrace molecular characterization of bioactive organotin species in saline waters, including both biological growth media and sea water. HPLC coupled automatically to a sensitive (ng to pg) graphite furnace atomic absorption spectrophotometer (GFAA) as a detector provided a versatile means for characterizing both monomeric and polymeric organotin species of fundamental significance to the program. With size-exclusion GFAA we showed the relationship of polymer kinetics during various OMP synthetic routes to ultimate incorporation of biocidal organotin moieties as a function of molecular weight, conversion, and molecular weight distribution. Recently, in unpublished work, we discovered a tin-119 NMR chemical shift relationship with the degree of polymerization of the OMP, and expect to establish tacticity and copolymer 1:1 fidelity by such methods. These aspects of the work have been successfully translated to applied Navy programs and are continuing. In a fundamental vein, HPLC employed in either ion exchange or reverse phase separations was shown to provide linear additive correlations of molecular substituent parameters obtained from independent literature values for ionic equilibria or partition coefficients, respectively, with thermodynamic data obtained directly from HPLC column capacity factors. Indeed, even geometric isomers of a given aryl or alkyl substituent can be so identified, or their resolution predicted, from independent substituent parameters, especially for cationic organotin species in water. For neutral organotins, the Hansch-Leo solvophobic parameter correlated to HPLC retention measurements presages an entirely new prospect for predicting cellular or membrane uptake and toxicity.

Completion of an automated purge and trap GC-FPD system designed to speciate both involatile, e.g., adsorbed or solvated ionic organotins, and volatile organotin species directly in aquatic samples has provided the first evidence for both TMT and methyltin hydrides in polluted estuarine environments. These data are highly relevant in light of our reexamination of the biomethylation of inorganic tin(IV) where we showed by GC-MS that both TMT and methyltin hydrides also occur in respirant gases above a *Pseudomonas* spp. isolated from the Chesapeake Bay. Publication of this work sparked world-wide activity and work of others now appearing generally confirms the biomethylation pathway. Nevertheless, the central question of biodegradation pathways of man-made organotin toxicants remains unresolved. We and others reported the presence of butyltins, for example, in harbor waters. In studies being concluded, we have examined the fate of a major biocide, tri-*n*-butyltin cation with both isolated bacteria and sediments from the Chesapeake Bay. Our bioaccumulation experiments, monitored both by HPLC-GFAA and GC-FPD, uniquely show that a trace organometal biocide can be tracked through uptake and removal from cells and any molecular transformations occurring can be quantitated *in vitro*. Though much more complex, similar results are anticipated from ongoing studies with viable sediments. We have established in this program the needs and means for extended research in the field of aquatic transformations and fate of organotins; the relatively simple speciation methodology generated in the program, now adopted by many laboratories, points to generating realistic modeling data for characterizing isomeric forms of key diagnostic organotin molecules involved in environmental tin transformations.

PHYSICAL CHEMISTRY OF SEAWATER

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Long-Range Scientific Objectives

This research program is designed to obtain information on chemical processes occurring in the ocean using the techniques of physical chemistry. The chemical reactions of primary concern in this work include solution equilibria, solid phase solubility, adsorption equilibria, and oxidation reactions.

Project Objective

This project is currently focused on studies of the physical chemistry of trace metals in seawater with particular emphasis on iron. Laboratory investigations are being made to develop a consistent set of complexation equilibrium constants which can be used to calculate the equilibrium speciation of iron(III) in marine environments at various temperatures, salinities, pressures, and pH. We have also studied some of the oxidation-reduction reactions of iron(II) and iron(III) in seawater. Field measurements of iron, copper, and vanadium are being conducted to provide analytical data which may be related to the physical chemical studies.

Current Status of the Project

Our recent studies of transition metal complexation equilibria have made extensive use of computer controlled spectroscopy and data analysis. A laboratory minicomputer has been interfaced to a Cary 17 recording spectrophotometer and a set of programs has been developed and refined during the past year to facilitate the acquisition, manipulation, and analysis of high resolution ultraviolet, visible, and near-infrared spectroscopic data. This capability has been applied to several chemical species of iron including Fe^{3+} , FeOH^{2+} , $\text{Fe}(\text{OH})_2^+$, $\text{FeB}(\text{OH})_4^{2+}$, FeCl^{2+} , and FeCl_2^+ . Recent measurements have been made to assess the effect of ionic strength on the Gaussian parameters that can be used to characterize the ultraviolet absorption spectrum of specific transition metal species. We have been investigating the use of anodic stripping voltammetry (ASV) to measure copper and its speciation in seawater. A theoretically based model has been developed and tested experimentally for ASV measurements by rotated disk thin film electrodes using copper, cadmium, lead, and thallium. A number of refinements have been made in the cobalt-APDC preconcentration technique used for atomic absorption measurements of trace metals in seawater. A set of samples were processed and analyzed for iron, cadmium, and copper in waters of the Sargasso Sea, the Slope Water, and along a transect across the continental shelf to New York City.

Significant Accomplishments

We have found that the ultraviolet absorption of specific chemical species of iron (and also of copper) can be accurately characterized by one or two Gaussian bands. This approach to ultraviolet spectroscopy provides a powerful means to deconvolute complex spectra and to investigate transition metal speciation. We have found that the rate of iron(II) oxidation is 100 times slower in seawater than would be

expected on the basis of fresh water and simple solution studies. The reduced rate of this reaction is not due to organic matter; it can be accounted for in terms of an ionic strength effect and of specific effects due to chloride and sulfate ions in seawater. Our studies have shown that anodic stripping voltammetry can be used at sea to provide low concentration measurements of copper in ocean water which are consistent with shore-based atomic absorption analyses. However, interpretations related to copper speciation in seawater using ASV are tenuous at best, and possibly not valid, because copper(I) acts as an intermediate in the electrochemical reduction step.

Future Plans

During the coming year we will continue our studies of the ultraviolet absorption of iron species and we will develop a system for high pressure spectroscopic measurements. The high pressure measurements will be used to investigate the extent to which iron(III) speciation varies with pressure and hence with depth in the ocean. The apparatus will consist of a specially constructed cell for measurements up to 500 or 1000 atm and a hydraulic system to generate the pressure. We will continue our studies of anodic stripping voltammetry measurements in seawater through a series of experiments to compare ASV and atomic absorption spectroscopy in relation to operationally defined metal speciation.

INTERACTIONS BETWEEN MICROORGANISMS, SOLIDS AND TRACE METALS

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Long-Range Scientific Objectives

Our long-range goal is the understanding of the adherence of bacteria to solid substrates and the interactions between bacteria, solids and trace chemical components of seawater. Results of the program should provide information applicable to problems of biofouling, corrosion, biological mineralization of organic matter in the oceans and the effect of bacteria on metal ion speciation in marine systems.

Project Objectives

To determine the effect of surfaces on bacterial metabolism, we have attempted to measure metabolic rates of bacteria attached to surfaces and living in suspension when presented with organic substrates attached and unattached to the surfaces. To determine the effect of trace metals on bacterial metabolism, and the presence of bacteria on the speciation of the metals in seawater media.

Current Status of the Project

During the last year we have a) studied the adsorption of glucose and glutamic acid on quartz and hydroxyapatite; b) measured metabolic rates of *Vibrio* being fed these substrates in the presence and absence of surfaces which alter the availability of these substrates to the bacteria; c) studied the effect of Cu^{2+} on the metabolic rates of *Vibrio*.

We have recently acquired a sorption calorimeter block for our existing calorimetry system which will facilitate our measurements of the metabolic rates of attached bacteria with changes in solution composition. Experiments are in progress to separate the cell components into (3) operationally defined fractions and to measure the copper ion uptake in each of these. We are continuing our work on the speciation of tin, using voltammetric and spectrophotometric methods, but have yet to incorporate the biological and solid components of the system.

Significant Accomplishments

Work on the measurement of the metabolism of marine bacteria has been presented at the Annual Meeting of ASM (Amer. Soc. Microbiol.), Dallas, Texas, 1981. Our work at present tends to belie the suggestion that surfaces greatly enhance the metabolism of microorganisms at low organic substrate concentrations. Work on the toxicity of free and complexed copper on a marine bacterium was presented at a NATO conference (Trace Metals in Seawater), Erice, Sicily, 1981. Papers on the inorganic speciation of tin in natural waters have been published.

DETERMINATION OF THE EFFECT OF COMPOSITION, STRUCTURE,
AND ELECTROCHEMICAL MASS TRANSPORT PROPERTIES ON
ADHESION AND CORROSION INHIBITION OF PAINT FILMS

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Long-Range Scientific Objectives

The primary objective is to develop a mathematical model of corrosion under paint films. Such a model would allow identification of critical factors affecting paint performance and eventually lead to improved paint formulations.

Project Objectives

To quantitatively describe the transport of ions and water through paints, and to determine the effects of physical structure and chemical composition on the transport properties of paints.

Current Status of the Project

Since 1979, nine paints meeting military specifications have been studied. Seven of these paints have been formulated with well known chemical compositions. A sequence of tests including dynamic mechanical, IR and UV spectroscopy, and small angle x-ray scattering was performed to determine the physical and chemical structure of the paints. At the same time, new apparatus and techniques were developed to measure the transport of ions and water through paint films.

In the past year, Hittorf experiments have been conducted to measure the migration of sodium chloride and water through paints. Improved "humidity chamber" apparatus and techniques have been developed to measure the diffusivity and solubility of water in paints attached to metal substrates. Five new mathematical models, developed to interpret the results of these experiments, have been used to deduce the physical form of coatings applied to metals. Preliminary experiments have been conducted to measure the hydraulic permeability of electrolytes through paints. These experiments were performed to obtain coefficients needed for the corrosion model. Also, experiments have been initiated to determine which components of paints have the greatest influence on water diffusivity.

Significant Accomplishments

Hittorf Experiments: The transference numbers and permeabilities of ions have been measured in Hittorf experiments. Most experiments were conducted on one type of polyurethane, but three other coatings have also been tested. The most significant finding was that when polyurethane is in NaCl solution, sodium and chloride ions do not carry all the current. The concept that ions other than sodium and chloride carry some current was proposed as a hypothesis in the last annual report. Only indirect evidence supporting the hypothesis was available last year. Since then, measurements have been made of both the sodium and chloride fluxes. These measurements confirm that the total current is greater than that attributable to sodium and chloride migration. Experiments have been conducted to determine which other

ions are carrying the balance of the current, but definitive results have not yet been obtained.

Water Solubility: The solubility of water in polyurethane has been measured with the humidity chamber apparatus. Eighteen samples of polyurethane film attached to gold have been tested. Water sorption by the paint was measured at five different relative humidities: 10%, 30%, 56%, 84%, and 98%. The temperature was 29°C. The results obtained with this apparatus are in agreement with those obtained by gravimetric methods using a Cahn electrobalance. The results may be interpreted in two ways; either the solubility increases as the coating thickness decreases, or a water-rich phase exists near the paint-gold interface. Work is continuing to determine which interpretation is correct.

Water Diffusivity: The diffusivity of water in polyurethane has been determined by measuring unsteady-state sorption. It has been determined that the diffusivity is not constant inside polyurethane paint. The diffusivity is now known to be a complex function of the water concentration and passes through a maximum between zero and 100% relative humidity (29°C). Model calculations suggest that the diffusivity may also be a function of position in the paint. Future work will concentrate on identifying features of diffusion generally applicable to paints. The paint components having the greatest influence on water diffusivity will also be identified.

Hydraulic Permeability: Hydraulic permeability experiments were conducted with five types of paint. The paints can be divided into two categories. Some paints exhibit very low permeabilities, on the order of 10^{-5} $\mu\text{l}/\text{psig}\cdot\text{cm}\cdot\text{min}$, and others have permeabilities 100 to 1,000 times greater. Some of the high-permeability paint films were nonuniform. Most of the flow was through small "weak" spots. A similar flow mechanism is possible for the low-permeability films, but this was not confirmed. All experiments were conducted in sodium chloride solution, and attempts were made to measure the salt rejection and the water flux. Only high-permeability films could be tested for salt rejection, and no rejection was observed. This result indicates that the "weak" spots in these coatings are relatively porous, and salt, as well as water, can penetrate the films.

THE REDOX KINETICS AND TRANSIENT PRODUCTS
OF SOME TRANSITION METALS IN SEAWATER

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Long-Range Scientific Objectives

To study the oxidation and reduction kinetics of transition metals (i.e. Fe, Cu, Co, Mn, Cr and U) in seawater and to elucidate the redox mechanisms operating in the natural marine environment.

Project Objectives

To develop a sensitive analytical technique for the measurement of the lower oxidation states of copper and iron in seawater and to use this method to determine oxidation rates of these metals under different conditions. Also, to develop instrumentation to study the short-lived chemical species involved in the redox processes of metals in seawater.

Current Status of the Project

1. During the current year of the project, the remaining components of a sophisticated stopped-flow and continuous-flow system have been purchased, assembled, and tested. This system is designed to provide rapid absorbance and luminescence spectral data of metal speciation changes and of redox products associated with light initiated reactions in metal-organic and natural organic constituents in seawater.
2. The oxidation of Cu(I) and Fe(II) in seawater are being examined in detail with respect to the rates of reaction and mechanisms involved.
3. Through a combined application of field and laboratory studies an attempt is being made to identify the marine abiotic processes which are potentially important in controlling the distribution between stable and unstable oxidation states.

Significant Accomplishments

1. Dynamic redox processes in seawater have been investigated through field and laboratory studies. From these studies, estimates have been made of the capacity of the upper mixed layer of the ocean to alter the non-dynamic equilibrium conditions which are proposed to exist there. Surface and depth profile measurements of hydrogen peroxide concentration and superoxide radical anion production rates in the western Atlantic Ocean and in coastal environments were used to make these estimates. Some aspects of these findings were presented at the Fall 1980 AGU meeting. Most of this work is being prepared for publication.
2. The oxidation rates of copper(I) and iron(II) have been measured in various media. Both metals exhibit slow oxidation rates in seawater relative to those found in distilled water at the same pH. It is apparent from spectroscopic evidence that differences in the coordination sphere of the metal cation are responsible for the observed differences in rate. These findings

are being prepared in the form of two publications: one describing the method used in the rate determinations and the other elaborating on the chemistry of the reactions involved.

PARTICULATE FLUXES

COMPOSITION, FLUX AND TRANSFER EXPERIMENTS (C-FATE):
TO STUDY THE PRODUCTION, VERTICAL FLUX AND DECOMPOSITION
OF OCEANIC PARTICULATE MATTER
DURING THE SEDIMENT TRAP INTERCOMPARISON EXPERIMENT (STIE)

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Long-Range Scientific Objectives

To develop a basic understanding of the factors governing the global distributions of chemical elements involved in the biogeochemical cycle through the study of oceanic particulate matter distributions, chemistry, reactivity and sedimentation.

Project Objectives

To deduce the rates of production, decomposition and sedimentation of particulate matter during the Sediment Trap Intercomparison Experiment (STIE) in the Panama Basin during two cruises in July and November 1979. A second objective was to relate techniques of estimating the vertical mass flux of particles using sediment traps and the Large Volume in situ Filtration System (LVFS). A third objective was to provide a biological framework for interpreting the scientific results of STIE. A final objective was to provide a basis of comparing samples collected over different time scales.

Current Status of the Project

1. The seagoing program was successfully carried out in 1979. Biological sampling consisted of primary productivity (C-14), vertical fluorescence, chlorophyll-a, and zooplankton (MOCNESS - Peter Wiebe from WHOI). Hydrography was characterized using XBT's and regular niskin sampling for Temperature, Salinity, and Nutrients (PO_4 , Si, NO_3 , NO_2 , and NH_3). Particulate matter distributions were determined using the Lamont Thorndike profiling and rapid rate nephelometers deployed during deep hydrographic casts, during LVFS sampling and at 600m on on sediment trap mooring. Particles were also sampled using 30 liter niskin bottles (0.4 μ m Nuclepore filters) and the LVFS. Sinking particle fluxes were estimated using surface tethered drifting sediment trap and LVFS deployments over one day time scale periods and using sediment traps attached to moorings for four months.
2. Coastal Zone Color Scanner (CZCS) data were collected periodically during the four month period of the experiment to assess the lateral variability in surface chlorophyll in the STIE area. Data tapes did not arrive at L-DGO until October 1981 but processing will be attempted on several images.
3. Laboratory analysis of samples has been largely completed.
4. We participated in the STIE workshop November 1980 and extended abstracts of presented papers have been published in WHOI technical report .
5. Scientific results of the project were presented in invited papers at the Gordon Conference in New Hampshire (August 1981) and at AGU (May 1980, December 1980).
- 6 We are preparing manuscripts to be published in a collected volume of papers next year.

Significant Progress

1. We have determined that primary productivity at the STIE site (5°N , 82°W) is controlled by light (cloudiness), nutrient content of the mixed layer (given by sea-surface temperature) and mixed layer depth. A model has been constructed which uses NOAA/NMFS (La Jolla) cloudiness, SST and DML data as input and shows factor of two to three times enhancement of productivity above typical values during roughly 10% of the time due to entrainment of cold nutrient rich water into the mixed layer due to mixing. We have also shown that phytoplankton production and zooplankton grazing are tightly coupled.
2. Nephelometer profiles suggested that resuspension and lateral mixing was an important source of particles to the water column below 1500 m. Rapid rate nephelometer data showed large particle (marine snow) distributions to be relatively uniform during LVFS deployments over the depth range of 100-530m on two days in early August. A factor of two variation in large particle abundance was recorded over a two week period by the nephelometer attached to the sediment trap mooring at 600m.
3. Moored sediment traps showed excellent intercomparison for designs that have been widely deployed in the ocean. Calculated mass flux based on LVFS data compared with the moored traps showed LVFS estimates to be factors of two - three high and low compared with traps for the depth interval 300-1000 m sampled during the July and November cruises. These differences were considered to be reasonable given the different time scales of sampling employed by the two methods. Comparison of vertical particle mass flux using floating sediment traps and the LVFS during the July and November cruises showed larger differences. Systematics of the LVFS data appear to be consistent with hydrographic and biological data of each cruise.

BIOGEOCHEMISTRY OF ORGANIC MATTER IN SEAWATER PARTICULATES AND SURFACE SEDIMENTS

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Long- Range Scientific Objectives

Our long-range objectives are to study the biogeochemistry of organic matter in seawater particulates and recently deposited sediments to better understand (i) the carbon cycle of the water column and benthos, (ii) the influence of organic matter on the geochemistry of mineral material nutrients, and trace metals, especially surface chemistry of particulates, (iii) the influence of organic matter on surface sediment resuspension, (iv) the interaction of organic matter and biological processes.

Project Objectives

To define the biogeochemistry of lipid compounds associated with large particles caught in sediment traps, the influence of biological processes in euphotic and mesopelagic zones on the lipid biogeochemistry and the relationship between particulate matter lipids and surface sediments.

Current Status of Project

Analysis of lipids in floating sediment traps and surface sediments from the Peru upwelling area near 15°S are nearing completion. Analyses of sediment trap samples from the PARFLUX E (Particle flux) moorings in the equatorial Atlantic have been completed and papers on the detailed composition of fatty acids, triglycerides, wax esters and sterol esters are completed. PARFLUX P (Pacific) mooring samples at 15°21'N 151°28'W are in progress as are the sediment trap intercomparison samples (S.T.I.E.) from the Panama Basin and the first VERTEX (Vertical Transport and Exchange Experiment) samples from moorings off Monterey, California in the California Current area.

Preparations for the second VERTEX experiment in the Equatorial Pacific off Mexico in November, 1981 are in progress.

High resolution glass capillary gas chromatography and gas chromatography/mass spectrometry computer systems analyses of samples are now moving from analytical development stages towards more routine analyses. Mathematical geochemical modeling of the data is in the initial stages.

The VERTEX experimental and sampling design has been oriented towards gaining maximum information about the relationship between biological processes in the upper 1000 meters of the water column and large particle lipid biogeochemistry.

Significant Accomplishments

We have demonstrated conclusively over the past eight years that early diagenetic processes in the surface sediments of diverse oceanic regimes - upwelling areas, near-shore U.S. coastal regions, abyssal areas of the Western North Atlantic -

can have a large influence on organic matter found in ancient sediments. Furthermore, insights into sources of organic matter and processes acting on organic matter can be gained from detailed analyses of individual lipid class compounds such as fatty acids, hydrocarbons, sterols and amino acids.

During the past two years, we have shown that even though large particles sink rapidly through seawater with little apparent change in total organic carbon content, the detailed analyses of individual organic compounds reveals marked changes in composition with increasing depth of sediment trap collection. The changes in composition observed reflect the many biological and geochemical processes which act on these large particles even though they sink rapidly through the water column.

PHYSICAL AND CHEMICAL PROPERTIES OF BIOGENIC SILICA AND ALUMINO-SILICATES
RELATING TO RATES AND MAGNITUDES OF RESERVOIR TRANSFER WITHIN THE OCEANS

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Long-Range Scientific Objectives

To produce well-defined models for a series of biogenic mineral systems which will quantitatively describe the fluxes of these materials to and from various reservoirs. In particular, dissolution and precipitation rates will be applied to the physical reality of the material relative to its chemical properties. The effects of oceanic pH, temperature and salinity ranges will be considered and included in the model.

Project Objectives

To understand better the effects of surface energy and particle size on biogenic silica dissolution rates; to determine the equilibrium concentration of dissolved aluminum with respect to a variety of aluminosilicates; to study the changes in surface morphology of biogenically precipitated silica using transmission electron microscopy.

Current Status of the Project

During the past year, both Mr. Vernon Asper and Mr. Scot Birdwhistell completed their M.S. theses while working as visiting students under ONR auspices at WHOI. It is expected that both students will publish part or all of their work but a definite time schedule has not been established. Mr. Asper has been accepted for graduate study at WHOI where he will pursue studies leading to a Ph.D. Mr. Birdwhistell has accepted an offer as a research technician at WHOI and will be working for Dr. Fred Sayles. Dr. David C. Hurd has accepted employment offered by Shell Development Company, Geological Engineering Section. No funds will be requested of ONR for this project in the foreseeable future.

Significant Progress

A paper describing Hurd's silica dissolution studies was given at Fall 1980 AGU and has been accepted for publication by the American Journal of Science. The ONR-sponsored pressure solubility work by James Griffin has been expanded and gone through one round of review for the American Journal of Science. A revised version will be re-submitted by December, 1981. The field study regarding aluminosilicate solubility as a function of pressure and temperature has been completed and the results compared to existing thermodynamic calculations.

BIOGEOCHEMISTRY OF ORGANIC NITROGEN COMPOUNDS
IN SEAWATER AND ON PARTICLES

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Long-Range Scientific Objectives

The objective of this research is to increase our understanding of the behavior of organic nitrogen compounds in the marine environment. Nitrogen is essential to the photosynthetic formation of life in natural waters and can control biological productivity through its role as a limiting nutrient. For this reason, the regeneration of nutrient N from organic nitrogen compounds is extremely important in the estuarine, marine coastal, and upwelling systems which play such a major role in world biological production.

Project Objectives

Immediate goals are to determine the sources of production, the transition behavior, and the ultimate fate of certain classes of organic nitrogen compounds. This study includes investigations of these compounds in seawater, sediments, particulate matter, and sediment trap material, since the geochemical cycle of organic nitrogen involves transport between these various geochemical reservoirs. An interdisciplinary approach should yield the most information since the transport and transformation of nitrogen is strongly influenced by biological, physical oceanographic, and geological processes.

Current Status of the Project

1. The Sediment Trap Intercomparison Experiment took place in the Panama Basin during July-December, 1979. We are currently completing the analysis of samples from traps which were deployed as part of S.T.I.E. These traps collected seven samples in time series providing an opportunity to look at fluctuations in particulate flux over relatively short (two-week) periods. And, since these time series samples remained in separated, closed containers during the entire 112-day deployment, we can directly assess the efficiency of sodium azide as a deterrent to microbial activity.
We are also analyzing samples from a large volume in-situ pump which was deployed at the same depth as the trap. There is some controversy over what sediment traps collect vs. what in-situ pumps collect since the percent organic carbon in the particulate matter collected varies greatly between the two sampling methods. Our analyses may help explain these differences since the nitrogen compounds being analyzed represent about 30% of the organic matter and will reflect possible sources for the material.
2. We participated in the interdisciplinary VERTEX (Vertical Transport and Exchange) program. The first cruise to the central California upwelling was successfully completed during August-September, 1980. Nine particle-interceptor traps (PITs) were deployed for two weeks at 50 m, 100 m, 200 m, 300 m, 500 m, 600 m, 700 m, 900 m and 1700 m. We also obtained a detailed vertical 2000-m water column profile of seawater and small (filtered) particulates. Phytoplankton and zooplankton samples were collected, and a

short-term zooplankton fecal pellet decomposition experiment was carried out.

We are currently preparing for the November 1981 VERTEX cruise to the eastern tropical North Pacific Ocean (ETNP). Productivity in this area is higher than that occurring off central California. The inorganic nitrogen cycle has been well studied in this area. A zero oxygen minimum exists with pronounced denitrification occurring in the upper 400 m of water. We will determine the effects of this rapid N recycling on the organic N compounds. Large particle fluxes in this area should be very different from other sites since most zooplankton do not enter anoxic areas and their fecal pellets would be excluded from the zero oxygen areas. Also, the activities of anaerobic bacteria in the oxygen minimum should affect the sinking organic matter differently than aerobic microbial activity.

Significant Progress

1. Amino acid analyses of sediment trap samples from the Peru upwelling area have been completed. A paper based on these results and on the PARFLUX E Site (equatorial Atlantic) results has been accepted by the Journal of Marine Research.
2. Preliminary results from the central California VERTEX cruise are being completed. The VERTEX sample collection is unique because for the first time we have representative samples from all of the major reservoirs of organic nitrogen in the upper 2000 m of water. After measuring the concentration of specific organic nitrogen compounds in these samples, we can determine which pathways of remineralization are most important and compare these calculations with the rough budget made for the Peru upwelling area. Also, the mechanism and relative rates of amino acid decomposition can be compared in the two areas. Since individual organisms collected in the PITs were counted, we will be able to directly assess the contribution of organic nitrogen made by these organisms for the first time.

NOBLE METAL BIOGEOCHEMISTRY

THE MARINE BIOGEOCHEMISTRIES OF THE PLATINUM METALS

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Long-Range Scientific Objectives

To investigate the inorganic and biologically controlled chemistries of trace metals and metalloids in seawater. Over the past several years we have studied the protonation and methylation reactions of such metalloids as arsenic, tin and antimony and the role of the biosphere in determining their concentrations and speciations in seawaters.

Project Objectives

To develop techniques for the analyses of the platinum metals (Group VIII), platinum, palladium, ruthenium, osmium, rhodium and iridium in seawaters. This is the last major group of elements which have never been analyzed in the oceans. Isolation steps to be evaluated include the use of reductive resins, of chelating resins and of electrophoretic deposition. Analytic steps will involve neutron activation analysis and laser-induced resonance ionization analysis.

Current Status of the Project

1. Using 17-day radioactive Pd-103, the efficiency of the commercially available reductive resins (Amboranes) for the uptake of palladium is being sought as a function of pH, flow rates and amount of resin in column.
2. Bismuth is being used as a surrogate for the platinum metals in some initial experiments where the Bi-210/Bi ratio is sought in isolates from very large volumes of seawaters and the Bi-210 is sought in small volumes of seawater. The bismuth seawater concentration is then computed. Present results suggest that the bismuth value in seawater has been overestimated by a factor of 100, perhaps as a result of laboratory or field contamination.
3. As a spin-off from our development of techniques for metalloids using hydride generation, we have developed a unique method for nickel analyses in seawater involving carbonyl generation. Nickel is reduced to the elemental form, and then the addition of carbon monoxide allows nickel to be volatilized into an atomic absorption apparatus. We are refining our procedures as well as developing techniques for Fe (II) and Fe (III) assays using the carbonyl generation technique.

Significant Progress

1. The speciation of antimony in oxic waters of Saanich inlet indicates the element exists primarily as Sb(V) with a few percent of Sb(III) which is thermodynamically out of equilibrium. Some Sb(III) production occurs in the photic zone. In the anoxic pore waters of the sediments, there is a large amount of

Sb(III), greater than 50% of the total antimony, which diffuses into the overlying waters. There is evidence for dimethylstibonic acid in the pore waters.

2. In three species of marine algae from San Diego Bay, only one has significant quantities of antimony (III), about 30%. This plant, Sargassum sp., illustrates an ability to produce reduced forms of the element and indicates a role of algae in the cycling of antimony in coastal waters.

EXTRATERRESTRIAL NOBLE METALS IN MARINE SEDIMENTS

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Long Range Scientific Objectives

To utilize noble metals, especially Ir, as tracers of the influx of extraterrestrial materials into the marine environment, to determine long term fluctuations in extraterrestrial accretion rates, to identify encounters with kilometer-sized objects and their possible effects on the environment, and to use anomalous sedimentary layers as time-correlative stratigraphic tools.

Project Objectives

To understand the marine geochemistry of Ir, to measure the Ir stratigraphy for the last 65 Ma, and to find and characterize anomalous horizons in marine sediments.

Current Status of the Project

Present efforts are concentrating on a search for anomalous horizons and determination of the background flux of extraterrestrial materials for the last 65 Million years. Analyses either completed or in progress are on sections from the Antarctic Ocean cores El3-3 and E21-17 as well as on about 30% of the 25 meter North Pacific giant piston core GPC3. Since GPC3 contains 65 Million years of abyssal clay stratigraphy, this core will be good for estimating long term variations in flux of extraterrestrial materials.

Significant Progress

1. Anomalous concentrations of Ir have been found in GPC3 at the Cretaceous-Tertiary boundary. This is the first location where this world-wide anomaly has been discovered in an oxidizing, abyssal environment. This finding supports the fish tooth stratigraphy which has been worked out by Doyle and Riedel on this core.
2. Anomalous concentrations of Ir and Au have been found in a 2.3 Million year old layer in El3-3. The initial analyses on this horizon have been published in Nature. We have discovered that the Ir is contained in a coarse-grained debris which must be derived from an atmospheric explosion or an oceanic impact.

Plans for the Future

We plan to characterize the debris from the 2.3 Million-year-old layer in El3-3 and to determine the magnitude of this accretionary event. Analyses for Ir, Au, Pt, Pd, Os, Ni, Fe and Cr are being conducted on bulk sediment and separated debris samples. Some debris particles have been sent to D.E. Brownlee at University of Washington for scanning electron microscope analysis and other particles have

been sent to K. Nishiizumi at UC San Diego for analysis of cosmogenic Mn⁵³. We will search for this horizon in a core from the South Atlantic collected about ~3000 km from E13-3.

SECTION V

OCEANIC BIOLOGY ABSTRACTS

PROGRESS REPORT ABSTRACTS



OCEANIC BIOLOGY



1 MARCH 1982

**OFFICE OF NAVAL RESEARCH
ENVIRONMENTAL SCIENCES DIRECTORATE**

SECTION V

Oceanic Biology Abstracts

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MARINE MAMMAL BIOACOUSTICS

THE BIOLOGY OF SMALL WHALES

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Work Unit No: NR 104-157

Contract N00014-77-C-0212

OBJECTIVES

Study the biology and natural history of small whales using aerial and ship surveys, radio telemetry, and new remote sensing technology. Special emphasis is placed on determining relationships between distribution, abundance, movements, and other behavioral and oceanographic parameters measured either directly or indirectly.

One subproject has been to investigate the potentials for research on cognition and communication in dolphins, including human-dolphin communication, and to hold a workshop to evaluate the field in conjunction with personnel from NOSC, U.C. San Diego, and other participants.

ABSTRACT

1. Research activity since 1977 has concentrated on two specific areas: (a) estimation of small whale population abundance and distribution (Globicephala macrorhynchus, Grampus griseus, Orcinus orca), and (b) developing remote sensing techniques for improved methods of assessment. Activity this past year has primarily been concerned with the latter, in conjunction with other agencies. We have continued the development and testing of a passive ship-towed linear hydrophone array (developed by Sea World, Inc., and tested by HSWRI, in conjunction with the U.S. Tuna Foundation). In six different field tests of this system in the Southern California Bight, both prior whales and common dolphins were located acoustically using this system at ranges of greater than five miles.

We are also discussing the availability of a satellite tracking system for use in our ONR program with J. Jennings of the National Marine Fisheries Service. This system involves monitoring distribution and movement patterns of marine mammals (to which radio-transmitters have been attached) by satellite tracking system. Preliminary testing of attachment on and monitoring of Stenella in Hawaii in June 1981 indicates that this method will provide valuable long-term information on distribution and movement patterns. We intend to determine the suitability of using this technology to monitor movement patterns of Globicephala throughout the year in the Southern California Bight.

Our third area of interest has been to evaluate remotely sensed oceanographic parameters (e.g., water color and sea surface temperature) and marine mammal density. Recent studies by NMFS and the Scripps Institute of Oceanography Satellite Visibility Laboratory have indicated correlation between high chlorophyll concentrations and increased density of some marine mammal species. We intend to evaluate this data as they apply to distribution and density of Globicephala, in conjunction with other active and passive monitoring of marine mammals distribution in the Southern California Bight.

2. As a subproject to this contract, in October 1980 a group of fifteen invited scientists gathered at HSWRI for a workshop entitled "Potentials for Research on Cognition Dolphin and Human-Dolphin Communication". The workshop was jointly hosted by Hubbs-Sea World Research Institute and the Program in Cognitive Science, University of California, San Diego. This workshop had as one of its tasks discussing the scope and organization of a State-of-the-Art Conference on these topics in the near future. Participants represented such diverse disciplines as bioacoustics, evolutionary biology, ecology, comparative psychology, cognitive psychology, linguistics, and dolphin training were represented. Specific areas of discussion at the workshop included (1) problems in characterizing the perceptual world of the dolphin, (2) comparative learning capacities of small cetaceans and primates, (3) the significance of brain size in relation to intelligence, and (4) theoretical problems in assessing cognitive capacities in dolphins, and (5) the significance of training techniques and methodologies. Participants recommended that a conference be held to discuss these topics in greater detail. Participants also recommended that broader-based funding policies be adopted to support further research in these areas. A transcript of the workshop is currently being edited for publication, and a future State-of-the-Art Conference is being planned.

PLANS FOR THE FUTURE

During the coming year we intend to apply satellite tracking systems to monitor pilot whale distribution and movements in the Southern California waters. We plan to (a) use available cetacean satellite tracking systems (through NMFS) to monitor pilot whale movements in areas of the Southern California Bight; (b) determine the relationship of remotely-sensed water color and surface temperature (by Nimbus VII satellite) to pilot whale distribution and behavior; and (c) acoustically locate and track and track pilot whales in the Southern California Bight, using a passive ship-towed linear hydrophone array. We also plan to continue organization of a state-of-the-art conference on dolphin cognition and communication.

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MAMMAL UNDERWATER ACOUSTICS

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ASSISTED BY

WORK UNIT NO. NR 104-131

CONTRACT N00017-77-C-0185

OBJECTIVES

(a) To systematically explore variables affecting the detection of submarine acoustic signals by mammals. Variables include characteristics of the signal, signal probability, payoff matrix, signal sequence, response task and memory, (b) to determine whether results cut across situations and species.

ABSTRACT

The results from our most recent experiments on how sea lions and fur seals process acoustic information having directional and qualitative cues suggest that when these pinnipeds are responding spatially, but to a paddle placed on the opposite side from where the sound source is located, they initially ignore directional cues and respond solely to qualitative cues. These results were unexpected and are opposite of those obtained when the animals were trained to go to the paddle located adjacent to the sound source. In the latter experiment, all animals responded solely to directional cues. Both the California sea lion and the northern fur seal have completed the experiment, and following their initial responses to qualitative cues, they then began processing both qualitative as well as directional acoustic information.

PLANS FOR FUTURE

We will analyze the data and prepare papers reporting experimental results.

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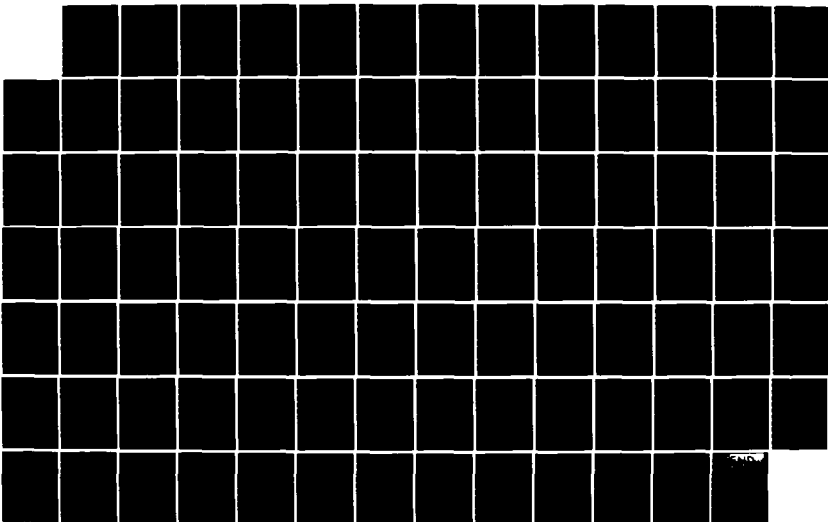
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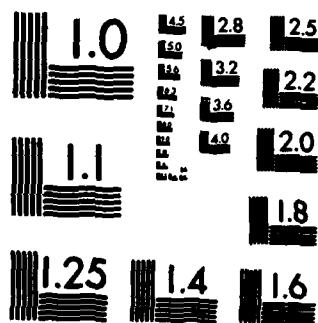
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COPY RESOLUTION TEST CHART

MARINE BIOLOGICAL SOUNDS

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ASSISTED BY Karen E. Moore

WORK UNIT NO. NR 083-004

CONTRACT N00014-79-C-0071

OBJECTIVES

The environmental biology of marine mammals, particularly of cetaceans, with special reference to their underwater acoustic activity, and their distribution.

ABSTRACT

The 2000-km radio track of finback whales between Iceland and Greenland provided new information on movements, speeds, feeding, dive-cycles, etc. The double tagging of one animal (behind blow hole and near fin) allowed assessments of different surface activities from the radio signals. Detailed analyses are underway.

Underwater sounds from finbacks were related to six routine activities of these whales. Consistent correlations indicated that their "higher frequency" sounds were used in communication with nearby finbacks, the "20-Hz" single pulses seemed to be for both local and longer distance communication, the patterned seasonal "20-Hz" sequences appeared to be courtship displays, the low-frequency rumble seemed to have agonistic significance, and non-vocal impulsive sounds were associated with surface feeding routines.

Low-frequency drum records from the Bermuda Sofar Station were surveyed for finback "20-Hz" pulse sequences and other similar patterns. These signals were a characteristic of the winter recordings. This data base has now been transferred to Woods Hole for analysis.

Local observations of cetaceans in Cape Cod waters provided good information particularly on the non-vocal sounds associated with surface feeding. The variety of species available for study in this area allowed good comparisons of activity and sounds of each species under similar situations.

Patterns of distribution and activity of right whales seen in near-shore waters were analyzed from our yearly observations since 1955. These data indicate year-round occurrence with increased visibility in April and May. The right whale sounds can be related to social activity and not to feeding or navigation.

PLANS FOR FUTURE

Low-frequency sound studies of whales in Cape Cod waters are planned, along with tests of 50 kHz sonar for tracking and target definition of these whales. Analyses are underway on the continuous radio track records from the Iceland finback, as well as on the 23 year Bermuda low-frequency drum recordings.

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(d) W. A. Watkins (1980), Acoustics and the behavior of sperm whales. Pp. 283-290, in: R.-G. Busnel and J. F. Fish, (Eds.), *Animal Sonar Systems*, Plenum Press, New York.

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(f) W. A. Watkins (1981), Radio tagging of finback whales - Iceland, June-July 1980. Technical Report WHOI-81-2, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, 46 pp.

(g) W. E. Schevill, K. E. Moore, and W. A. Watkins (1981), Right whale, Eubalaena glacialis, sightings in Cape Cod waters. Technical Report WHOI-81-50, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543, 16 pp.

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UPPER OCEAN BIOMASS STRUCTURE

BIOLUMINESCENCE MECHANISMS

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WORK UNIT NO. NR 104-386

CONTRACT N0014-75-C-0242

OBJECTIVES

To understand the following aspects of marine bioluminescence:

- (1) cellular processes regulating luminescence
- (2) membrane and neural regulation of luminescence
- (3) behavioral uses of bioluminescence by marine organisms.

ABSTRACT

INSTRUMENTATION DEVELOPMENT: An image intensifier system with a light gain of 10^6 has been completed and is in use. It is arranged for imaging both microscopic and macroscopic bioluminescent sources with data registration on TV tape. Acquisition of a fast spectrometer is anticipated within the next few months. Preliminary tests with this equipment resulted in excellent spectra from single flashes of single cells of Pyrocystis.

PORICHTHYS BIOLUMINESCENCE: Investigation of the dietary basis of luminescence induction continues. We are currently in a second and final season of assaying variation of luminescence capability in larval and juvenile Porichthys. We have been able to completely discharge the luminescence capability of juveniles and then restore it by feeding a locally available luminescent crustacean, Vargula tsujii. These experiments, together with our previous work on adults, strongly argue that this fish requires an exogenous luciferin source throughout its range if it is to be luminescent.

GNATHOPHAUSIA: Feeding experiments on laboratory maintained animals do not presently indicate a dietary requirement for a specific luciferin, contrary to earlier suggestions. Rather, the luminescence defect of specimens held for long periods in the laboratory seems nonspecifically related to nutritional state.

SERGESTES COUNTERILLUMINATION: Using the new image intensifier system, direct imaging of counterillumination is under study. This procedure permits monitoring individually of the 5 organs of Pesta. They are found to operate in close mutual synchrony while responding to variations in background illumination. Although our previous studies, conducted with photomultipliers, showed an excellent match of total luminescence with background illumination levels, these direct imaging experiments do not indicate that luminescence effectively eliminates the silhouette as viewed by organisms with reasonably good physiological optics.

Investigation of visual physiology of Sergestes as related to its bioluminescence

has commenced with study of the area and intensity requirements for downwelling light to trigger counterillumination. Moving shadows and small spots of light in the dorsal visual field shows that counterillumination may occur to light spots occupying only a very small part of the visual field, behavior that would seem to be maladaptive.

A study of the role of the statocysts in eye and luminescent organ orientation has been completed. At environmentally typical light levels, eye position is controlled, not by the direction of perceived light, but by the statocyst gravitation signal. Similarly, both the anterior and posterior organs of *Pesta* are positioned with reference to statocyst signals. Investigations of long-term light effects on visual function continue.

PYROCYSTIS: Work on the problem of luminescent microsource coordination in the two characteristic flash types of this dinoflagellate has been completed. Other properties of microsources are under study with the new image intensifier system. These include their numbers, capacity for repetitive activity, fatigue characteristics, and their behavior during the daily cycle. In collaboration with Prof. B.M. Sweeney, a coordinated EM and IIT study of microsources is in progress with the goal of obtaining definitive EM characterization of the microsources.

Using the single cell stimulation system, a study of the radical changes in excitability associated with temperature variation is in progress.

OTHER BIOLUMINESCENT SYSTEMS: Previously ONR-supported insect bioluminescence studies continue to be published.

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SUBMITTED

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- Latz, M. and J. Case. Gravitational cues in counterillumination behavior of *Sergestes similis*.
- Case, J. Visual integration in synchronic flashing of a firefly, *Pteroptyx tener*.

ACOUSTICAL TECHNIQUES FOR THE STUDY OF NEKTON AND ZOOPLANKTON AT
WATER TYPE BOUNDARIES AND OCEAN FRONTS

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ASSISTED BY B.G. Heist and T.K. Stanton

WORK UNIT NO. NR 083 503

CONTRACT N00014-79-C-0703

OBJECTIVES

To study nekton and macro zooplankton at the interface between different types of water. Studies indicate that volitional behavior is involved in the distribution of animals across the interface. Since the distributions are space and time dependent, we need rapid means of determining fish densities across the interface. Experience shows that acoustics is a powerful tool and that the poor resolution of common fisheries type sonars is inadequate. Objectives: Year 1: To build an additional receiving system for our Simrad 120 kHz sonar. The new receiver is to have high resolution multibeam capability. Year 2: To use our high resolution sonar to study mysis, alewife, and smelt at fronts on Lake Michigan or Lake Superior. Years 3 and 4: To take our high resolution system to the ocean and measure fish densities at the interfaces between water types.

ABSTRACT

This report describes our progress in the first phase of the project--instrumentation. The equipment is part of the development of advanced and simplified techniques for remote acoustic measurements of fish. These techniques are to be used in the correlation of (fish) biomass distributions to oceanic thermal fronts.

We decided to use a hybrid combination of analogue and digital processing of the acoustic data and to display certain results in real time. The purpose of the analogue processing was to reduce the data rate and volume so that small computers such as the PDP 11 23 and the APPLE II can handle the information. The PDP 11 23 was chosen for "number crunching" and the APPLE was chosen for graphic display. For duplication of critical components of the digital system, we ordered two nearly identical systems.

The APPLE II computers were received at the first of the year. Preliminary tests of the APPLE showed that it might be possible to digitally process the echo integration data from the sonar. Accordingly, Powell of the seismology group and Stanton of our group have developed the software to do this. They have written most of the program in FORTH, a high-level computer language that is particularly convenient for "general housekeeping" controlling input, output, A/D conversions, stacking, etc. The actual high speed data sampling is done in assembly language. (For more details see Stanton and Powell, 1981.)

Two techniques are in use for measuring the number of fish per unit volume (fish density): (1) measurements of the echo amplitudes of the same fish as observed with a wide and narrow beam transducer and (2) measurements of the probability distribution of echo amplitudes. When used with common echo sounding sonars having beam width of 10° to 30° , both of the techniques require rather low fish densities because individual echoes are required. We believe that we can use a sonar having many narrow beams (2°) to count the fish directly. Since the beams

are smaller, the maximum fish density can be larger and still receive individual echoes or each beam.

A trawl transducer was built by modifying a commercial echo sounder (200 kHz). The transducer and receiving circuits were placed on the head rope of a mid-water tucker trawl. The trawl (4 m x 3 m) will be aimed by the use of two thermistors and a depth sensor. The sonar signal is displayed by a chart recorder to determine the position of fish relative to the trawl. This will allow us to aim the trawl at the fish school of interest.

The DIMUS (Digital Multibeam Steering) Receiver Sonar System has been constructed. It is designed to form 64 stereo acoustic beams from an array of 16 transducer elements. Preliminary beam pattern measurements in a pool were made and the beam patterns compared satisfactorily with our theoretical calculations.

The research group is on a two-week cruise in Lake Michigan to survey the acoustic biomass in the lake (August 23 to September 5, 1981). When they left, all gear was field tested and operational.

PLANS FOR FUTURE

The major instrumentation phase is complete. Future work will concentrate on making acoustic measurements of nekton and plankton, Years 2, 3, and 4 of our objectives.

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CENTRAL GYRE RESEARCH PROGRAM

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ASSISTED BY P. Walker

WORK UNIT NO. NR 083-005

CONTRACT N00014-80-C-0440

OBJECTIVES

(a) To describe the structure of a climax epipelagic community; (b) to document the small and large scale structural stability in space and time; (c) to understand the environmental factors and biological interactions which regulate the community.

ABSTRACT

Since 1968 we have been studying the physical and biological structure of the epipelagic community of the North Pacific central gyre. This is a very old and stable community and the species structure of the phytoplankton and macrozooplankton appears to have reached a stable, self-regulating state, making this an ideal place to test modern ecological theory and to determine the physical and biological processes (disturbance, competition, predation, etc.) regulating the system.

Recent studies have been concerned with (a) examining the temporal stability in the depth distributions and species structure of the phytoplankton, (b) a comparison of small and large scale heterogeneity in species structure of the copepods in order to assess the role of disturbances in the regulation of species structure, (c) a comparison of heterogeneity in nutrients, chlorophyll, primary production and macrozooplankton biomass on differing spatial and temporal scales in order to assess the role of environmental forcing on various scales, and (d) an analysis of the physical and biological data collected on expedition FIONA (August 1980) to show how spatial gradients in the physical structure of the water column affect vertical mixing, nutrient inputs to the euphotic zone, and the carrying capacity of the system.

PLANS FOR FUTURE

Data interpretation and manuscript preparation will continue to occupy a large fraction of our time. Manuscripts are in preparation on each of the above subjects. The biological data from FIONA show a strong spatial gradient in carrying capacity (chlorophyll, production and macrozooplankton) and we are now attempting to relate this gradient to the physical structure of the water column. The patterns in patchiness and species structure over a many year period at one locale are now being related to large-scale spatial patterns.

CURRENT REPORTS AND PUBLICATIONS

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INVESTIGATION OF PLANKTON PATCHINESS RELATIVE TO HYDROGRAPHY AND BIOLOGY

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WORK UNIT NO. NR 083-005-67

CONTRACT N00014-75-C-0152

OBJECTIVES

(a) To study nearshore vertical and horizontal zooplankton patchiness on scales of tens of centimeters to kilometers, using 1.2 MHz reverberation as a measure of zooplankton distributions, (b) to determine the correlation between this reverberation, the distribution of chlorophyll and water temperature structure, and (c) to determine the correlation between large individual targets detected in a 1.2 MHz dual beam system and reverberation levels around those targets.

ABSTRACT

Preliminary analysis has been completed on data collected from a number of instruments at one site to look at the scales and causes of small scale zooplankton patchiness in nearshore waters. The experimental design consisted of three 1.2 MHz dual beam transducers on the bottom, pinging up at water flowing by; a vertical, 32 element thermister string; a current meter; and a flow-through fluorometer intake hose suspended at the local chlorophyll maximum depth. Horizontal data resolution was on the order of 0.1 m in a 10 cm/sec current for all instruments. Vertical acoustic resolution was 0.08 m, vertical temperature resolution was 0.5 m. Data was recorded for 2.5 hours. The study site was in 20 m of water, 4 km off Imperial Beach, California, and occupied one day in December 1980 during clear weather and glassy seas.

Acoustic data from the experiment was of two types. (a) Range gated reverberation from the narrow (0.3°) beams was used as an estimator of overall zooplankton concentrations, with which it had been highly correlated to copepod concentrations in laboratory tank studies. (b) Simultaneous acoustic returns from the narrow and wide beams (1.0°) from each transducer were used to detect large, individual targets with absolute target strengths equal to or greater than -90 db at 1.2 MHz. Laboratory tank work linked this target strength to copepods, mysids and euphausiids 2 mm to 3 mm long.

Three results are evident in the field data:

- a) Vertical reverberation profiles show no obvious correlation with the weak, vertical thermal or fluorescence structure that existed at the study site. Reverberation levels decrease with depth.
- b) Variability in horizontal reverberation is greatest near the surface, decays smoothly with depth, is not reflected in the temperature and appears to be the result of a previously unnoticed, 16 second surface wave.
- c) Individual acoustic targets equal to or greater than -90 db were found predominantly in the upper 10 m and were surrounded by reverberation levels

significantly higher than both the water column mean and the mean per depth to a distance of 3 m.

PLANS FOR THE FUTURE

Two avenues of work suggest themselves:

- a) Affix the multi-instrument package to an existing underwater power and data cable strung out from Scripps pier to gather long time data series under different seasonal conditions, and pump sample water at the study site to determine zooplankton species and acoustic biasing due to bubbles, detritus, etc.
- b) Use the dual beam transducer system to look at the onset and extent of vertical migration and other behavior in zooplankton populations in large laboratory tanks.

MECHANISMS OF BIOLUMINESCENCE: KINETICS-SPECTRAL AND BIOCHEMICAL
PROPERTIES AS A PROBE OF MARINE COMMUNITIES AND INTERACTIONS

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WORK UNIT NO. NR SRO-015

CONTRACT N00014-80-C-0066

OBJECTIVES

(a) Long-term color and temporal measurement of oceanic bioluminescence occurring naturally in the coastal water column from 200 m depth to the surface; (b) color and temporal measurement of luminescent emissions of commonly occurring midwater organisms; (c) use color and temporal features of known organisms as a luminescent indicator of their presence and behavioral mode in the ocean; (d) log information on presence and behavioral activity of such organisms, as well as ancillary environmental data; (e) use this data to create a model that predicts the occurrence and behavior of luminescent organisms in these waters.

ABSTRACT

A 3-point taut line mooring holding a 2360-kg float 18 m below the sea surface and directly over the axis of Scripps Canyon has been erected. This station configuration allows placement of an instrument package to a depth of 200 m while avoiding damage to, and loss of, instruments by turbidity currents. Power (220VAC) and data (digital) are transmitted between lab and field station via a 1400-m submarine cable. Sixteen data channels are available, each capable of transmitting 1.5 samples per millisecond. The data are logged into a Varian 620 L data acquisition computer. A photometer designed and calibrated by the Visibility Laboratory at Scripps Institution of Oceanography is about to be installed below the subsurface float at depths to 200 m. This photometer will collect data on the temporal features of radiance due to bioluminescence and sunlight.

Further developments scheduled for the sea station are the installation of a subsurface winch below the center canyon float and a submersible polycrometer.

We have developed a 6-type polycroic polycrometer that is now recording color and temporal data on bioluminescent emissions from pelagic organisms in the laboratory. The signal is processed through a 6-channel ADC and stored; the data can be viewed as a 3-dimensional surface which can be displayed or printed. These surfaces constitute our first records of the bioluminescent signatures of organisms.

PLANS FOR THE FUTURE

We shall continue the development of our luminescent signature files for the commonly occurring luminescent organisms in the vicinity of our study site, and reduce these signature data files to generalized algorithms that are taxa specific. The algorithms will then document the presence of organisms when they luminesce in view of the sea site photometer. We hope to extend our study area to deeper water and eventually to use free-floating photometric arrays that will record, nondestructively, many of the migratory, behavioral and ecological features of the luminescent organisms of the mesopelagic and generate predictive models of these features.

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BIOLOGICAL AND ACOUSTICAL STUDIES OF MIDWATER ANIMALS

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ASSISTED BY J.M. Willis, D.L. Stein and T. Miller-Way

WORK UNIT NO. NR 083-102

CONTRACT N00014-79-C-0004

OBJECTIVES

(a) To produce a better understanding of the distribution and ecology of micronekton and nekton of the North Pacific Ocean, including horizontal and vertical variations in abundance, zoogeographic distributional patterns, and food-web relationships; (b) to improve our ability to sample these communities using nets and acoustical techniques.

ABSTRACT

Similarities in the composition of mesopelagic fishes from the Central Northeast Pacific demonstrated the existence of three basic faunal regions in this area: Subarctic, Central gyre, and "southern" or Hawaiian. Boundaries between regions were correlated with oceanic fronts.

We have compiled an extensive set of data on mesopelagic fishes of the Subarctic Pacific, using midwater trawl collections over the period 1951-1978. These data have been coded for computer analysis. Preliminary results, including species occurrence maps, will be produced soon.

A cruise is planned for September 1981 to study diel migration of mesopelagic micronekton off Oregon. An arcer system will be used to measure resonance structure of migrant and non-migrant scattering layers. Directed sampling in selected features will be conducted with our new 100 m² pelagic trawl. Simultaneous acoustical data will permit comparisons of acoustical and trawl estimates of variances.

Preliminary acoustical measurements of abundance of micronekton along an 11 km transect revealed a complex structure, both horizontally and vertically, over almost all resolvable scales. Both the deep (ca. 300-500 m) and shallow (ca. 175 m) layers displayed scattering minima during the transect, suggesting that discrete aggregations occur in oceanic waters of Oregon.

Studies of the distribution, reproduction, growth, and feeding habits of the stomiatid fish Tactostoma macropus are nearly complete. This northeastern Pacific transitional species is uncommon in Subarctic and western Pacific waters. Interestingly, eggs and larvae are usually found > 200 km offshore despite the presence of mature adults closer to shore. Mature individuals are apparently non-migratory, residing at 400-500 m; small (< 250 mm) individuals undertake distinct diel migrations.

Analyses of catch data and stomach contents of nekton caught in gillnets in the Gulf of Alaska have been completed. Salmonids were the most abundant nekton except at the southernmost station (48°30'N), where pomfret (Brama japonica) was abundant. Most species of salmon preyed heavily on euphausiids at night but, in

general, squid were the most important prey. Squid were common prey for pomfret and sharks as well.

We began fabrication this year of an acoustical imaging device for use in analysis of net avoidance. This net sonar is intended to allow measurements of scatterer locations and movements up to 200 m in front of various nets.

As part of our development of multiple-frequency assessment techniques for micronekton, we have measured target strengths and polar scattering patterns of live euphausiids. A model incorporating frequency, size, and orientation dependence is being developed.

PLANS FOR FUTURE

We plan to continue our zoogeographic and ecological studies of micronekton and nekton of the North Pacific using data on hand. Net avoidance from an IKMT will be measured with our net sonar in 1982. We expect eventually to evaluate avoidance from our 100 m² pelagic trawl as well. A joint acoustical survey/directed net sampling cruise is planned to study the horizontal distribution of sound scattering animals off Oregon in 1982.

CURRENT REPORTS AND PUBLICATIONS

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(b) J. Fisher and W. Pearcy (submitted), "Distribution, reproduction, growth and feeding of the mesopelagic fish Tactostoma macropus Bolin (1939)." *Fishery Bull.*

(c) C. F. Greenlaw, R. K. Johnson and T. Pommeranz (1980), "Volume scattering strength predictions for Antarctic-krill (Euphausia superba Dana)." *Meeresforsch.* 28:48-55.

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(h) W. G. Pearcy, C. F. Greenlaw and T. Pommeranz (submitted), "Assessment of euphausiid abundance: comparison of five nets and 120 kHz acoustical measurements." *Biol. Oceanogr. J.*

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STUDIES OF OCEAN VOLUME REVERBERATION
AT HIGH ACOUSTIC FREQUENCIES

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OBJECTIVES

(1) To improve our capability to measure and understand zooplankton distribution and dynamics by applying ultra-high frequency acoustic technology to the problem of measuring absolute zooplankton abundance by size classes. (2) To interrelate the acoustically derived information on zooplankton distributions to temperature structure and chlorophyll distributions.

ABSTRACT

During the past four years, we have determined that small zooplankton, e.g. copepods as small as 0.1 mm, can be detected at sea using ultra-high frequency acoustics (0.5 to 3 MHz). Further, the quantitative analysis of the acoustic scattering has revealed complex structure in both the horizontal and vertical dimensions. These measurements have been correlated with variability in zooplankton collected with a high volume pump at the same time and place as the acoustic measurements. Acoustically directed sampling of millimeter size zooplankton can now be routinely accomplished. Associations of the acoustically detected zooplankton structure with temperature structure, chlorophyll profiles, anchovy larvae and the depth distribution and thickness of fish schools have also been noted.

Results of our work includes refinements to scattering models which relate observed acoustic scattering and the scattering projected on the basis of zooplankton collected by a plankton pump. Regressions relating abundance and size of the collected zooplankton, through scattering models, to observed scattering have confirmed our hypothesis that the dominant scatterers in our experiments between 0.5 and 3 MHz have been zooplankton. Achieving an in-depth understanding of this process and its parametric sensitivities was essential before approaching the more difficult inverse problem--deriving zooplankton size-abundance distributions from multi-frequency acoustic measurements.

Recent results include the measurements of the target strength of individual zooplankters at a range of frequencies from about 200 kHz to 10 MHz. Copepods and other zooplankters were tethered in an experimental aquarium above the acoustic transducers and their echoes were recorded. Over 2,000 separate echoes were recorded from live organisms in the aquarium which was filled with seawater from the area of their capture. While copepods of varying sizes were the principal target organisms, echoes from euphausiids, ostracods, larvaceans,

chaetognaths, ctenophores, pteropods, eggs, and larvae were also recorded. Preliminary assessments of this data are in agreement with the scattering models presently being used.

We have also continued work on the inverse problem as it applies to our attempts to extract zooplankton size from acoustic scattering data for multiple frequencies. A rigorous mathematical proof of the uniqueness of the solution of this problem has been developed.

Progress in the development of our up-dated measurement and data-acquisition systems has occurred in three different areas. (1) We are increasing the number of frequencies from four to 20 frequencies (100 kHz to 8 MHz), have re-designed and tested transmitters and receivers, and have written much of the software for the new data logging system. (2) We have procured and tested a new submersible pump for fluorometric measures of chlorophyll, a bongo net system, a new flow meter for the high-volume zooplankton pump, and a new conductivity probe. (3) We have further tested our surplus underwater vehicle and have modified the control systems to allow testing of the towed vehicle response.

PLANS FOR THE FUTURE

During the current year we are concentrating our efforts on (a) completing and testing the second-generation data-acquisition and measurement system (20 acoustic frequencies, ancillary sensors, shipboard data gathering and storage), (b) analysis and processing of the target strength data from individual organisms, and (c) continuing analysis and preparation for publication of results from prior cruises using the first-generation measurement system. Long-term plans include operation of the second-generation system to (a) extract size and abundance information on the zooplanktonic scatterers and (b) to utilize this system in various study areas to determine the interrelationships between the zooplankton distributions and distributions of temperature, salinity, and chlorophyll.

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(b) D. V. Holliday (1980), "Use of Acoustic Frequency Diversity for Marine Biological Measurements," in Advanced Concepts in Ocean Measurements for Marine Biology, F. P. Diemer, F. J. Vernberg, and D. Z. Mirkes, eds., Belle W. Baruch Library in Marine Sciences, No. 10, Univ. of S. Carolina Press, pp. 423-460.

(c) R. E. Pieper (submitted), "Quantitative estimates of euphausiid biomass determined by high frequency acoustics," Biol. Oceanography.

CELL DIVISION FREQUENCY AS A MEANS OF ASSESSING
NATURAL POPULATION GROWTH IN MARINE PHYTOPLANKTON

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OBJECTIVES

The goal of this research is to evaluate cell division frequency (CDF) as a means of determining in situ growth of natural populations of marine phytoplankton. Special emphasis is placed on: (a) establishing cell division frequencies for natural phytoplankton populations in coastal Gulf of Mexico and Beaufort, N.C. waters; (b) evaluating various culture methods and choosing appropriate algal species for comparing the CDF method with conventional cell count techniques; and (c) testing the CDF method relative to selected environmental variables in culture.

ABSTRACT

The first year objective of sampling natural populations of marine diatoms and analyzing for in situ growth rates was begun in June, 1980 at the Duke Marine Laboratory in Beaufort, N.C. Two questions were asked at the beginning: Can field-collected marine diatoms provide adequate nuclear division figures for mitotic index calculations? Can selected marine diatoms provide statistically significant growth rate data from mitotic index information?

Plankton samples were collected using a conventional 25u net. Four centric marine diatoms were selected on the basis of availability and size, Coscinodiscus radiatus, Guinardia flaccida, Rhizosolenia alata and Rhizosolenia styliformis. Samples were collected every two hours during seven separate 24 hour periods. Collections were immediately fixed and processed for DNA staining using aceto-carmine. Selected diatom species were carefully studied for morphological details characteristic of interphase and the various division phases. Cell division frequency was based upon the number of cells found in some phase of division (prophase through daughter cell production) versus the number of cells not in division (interphase). The resulting percentage of dividing cells generated the mitotic index or CDF for a given sample.

For each two hour sample period, 10 slides were prepared and the entire coverslip area was observed for cell division activity. Random block designed statistics applied to the data showed that no significant differences in CDF existed among the 10 slide counts for selected diatoms for each and all seven day samplings. Cell division frequency analysis depicted a bimodal curve in cell division activity during each of the seven sampling days for both Coscinodiscus and Guinardia, while a subtle sine wave activity was observed for both species of Rhizosolenia.

The CDF data for individual two hour sample times encompassing all seven sampling days were also analyzed statistically. No significant differences were observed for each sample time and sample day. The results appear to answer

affirmatively the first question. There is one exception in that CDF data points for the species Rhizosolenia styliformis appear greatly scattered during most of the sample days. Lack of adequate cell numbers in samples appears to be the basis for this discrepancy.

The CDF data were then applied to accepted growth rate formulae to generate: (a) percentage of population dividing, (b) specific growth rate and (c) doubling time for population. Results showed growth dynamics for given diatom species were similar over the seven day sample period. Data points are not widely scattered and show that the CDF method of quantifying in situ growth rates is statistically sound. On conditions that an adequate number of cells is available for counting, the technique shows considerable promise.

Specific growth rates generated from the CDF method showed values of 0.24-0.27 for Coscinodiscus radiatus, 0.20-0.23 for Guinardia flaccida, 0.04-0.12 for Rhizosolenia alata and 0.07-0.14 for Rhizosolenia styliformis. Doubling times for these species were 2.4-2.8 days, 3.0-3.5 days, 5.9-8.8 days and 7.5-10.6 days respectively. These values are well within the limits for marine diatoms as shown by others in the literature.

As part of our second year objectives, we are presently culturing appropriate marine diatoms. The test species will have to demonstrate adequate growth in defined seawater media, be readily available in the field or from culture collections and possess attributes lending toward rapid detection of cell division. A recently isolated Epithemia sp. is demonstrating all the qualities needed as a test organism. Isolation and growth studies with other species are also being carried out at this time.

PLANS FOR THE FUTURE

We plan to continue culture studies of selected marine diatoms to allow comparison of the CDF method with the absolute cell counts. Flask-grown species will be sampled every two hours and treated by both of these quantification techniques as a means of testing the accuracy and time-saving potential of the CDF method over traditional cell counts.

CURRENT REPORTS AND PUBLICATIONS

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MICROBIAL PRODUCTION OF NONCONSERVATIVE
GASES IN OCEANIC SURFACE WATERS

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OBJECTIVES

To investigate the involvement of particulate-associated microorganisms in the production of the reduced trace gases N_2O , H_2 , CH_4 , and CO in the upper nepheloid layers of the open ocean.

ABSTRACT

Profiles of dissolved gases (H_2O , N_2O , CH_4 , and CO) in a number of oceanic areas have often exhibited concentration maxima at or around the pycnocline. Shallow nepheloid layers are also found at these depths because of the increase in buoyancy occurring there. The gas maxima frequently coincide with ATP maxima and infrequently with chlorophyll maxima. This has led to the hypothesis that the source of at least some of the dissolved gases is biological and associated with particulate matter in shallow nepheloid layers. The investigation of this involvement has formed the basis of the current research.

Three major cruises have been undertaken which have covered portions of the Eastern Tropical North Pacific (ETNP), the Mediterranean Sea, the Caribbean Sea, and the Gulf of Mexico. On each cruise, nepheloid layer water was concentrated, dispensed into 50 ml serum vials, purged of oxygen, and incubated. Headspace gases were periodically withdrawn and analyzed by gas chromatography (GC). Half of the concentrated water samples were filtered through 0.2 micron Nucleopore filters prior to addition to the serum vials. A portion of both filtered and non-filtered water samples received nutrient supplements and/or other treatments.

Nitrous Oxide- C_2H_2 was added to half the samples to block reduction of accumulated N_2O . N_2O producing organisms were present in water taken from every station sampled during each of the three cruises. Net N_2O production (non-filtered minus filtered) was observed only when in situ nitrate levels were high e.g. ETNP or when samples were nitrate supplemented. (Filtered samples produced negligible N_2O and some nitrate supplemented samples that did not contain C_2H_2 produced net N_2O). The origin appears to be an anaerobic, non-photosynthetic, biological process.

Hydrogen-In the Mediterranean Sea, non-filtered, non-supplemented samples

produced low levels of net H_2 in the Eastern Basin and increased as sampling proceeded westward. Maximum H_2 production was measured just east of the Strait of Gibraltar within three (3) days of sample collection. Nutrient supplementation increased H_2 production in some instances. Filtered samples produced negligible H_2 even when nutrient supplemented. Sample depths correlated well with particulate, ATP, and H_2 maxima but not with chlorophyll-a maxima. The production appears to be the result of an anaerobic, non-photosynthetic, biological process associated with particulate matter. Similar results were obtained in the Caribbean Sea, although no consistent geographic trends were observed.

No consistent patterns of CH_4 and CO gas production have been measured.

PLANS FOR THE FUTURE

One major cruise is planned for CY 1982, during which we intend to occupy only a few stations but remain on each station for 3-4 days each. Multiple samples would be taken at each station in the surface nepheloid layers so that we may study the temporal and diurnal variations, (time, nutrients, producing organisms, depth of sampling relative to nepheloid layers, etc.) We have requested an anaerobic culture and incubation chamber with which to isolate organisms and determine growth and gas production characteristics.

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(c) S. J. Schropp and J. R. Schwarz. 1981. Shallow Water Hydrogen Production in the Mediterranean and Caribbean Seas. (In preparation)

PATCHINESS IN OCEANIC BIOLOGY AND PHYSICS

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CONTRACT N-00014-79-C-0200

OBJECTIVES

We are attempting to produce detailed descriptions of abundances and spatial distributions of organisms on very fine spatial scales (down to about 10 cm), to correlate those distributions with physical oceanographic fine-structure, and to use the distributions of organisms to study aspects of turbulent processes in the upper ocean which the physical oceanographic community is unable as yet to measure directly.

ABSTRACT

We have developed a towed video/CTD system which simultaneously measures fine-scale distributions of organisms (categorized as to size-frequencies), T, S, chlorophyll-a fluorescence, volume reverberation at 3 MHz, and other environmental parameters. Organisms are recorded by video camera, using strobed lighting at right angles to the camera's line of sight. Video pictures and all environmental data are recorded on a video tape recorder ('VTR'), as well as displayed in real time on a monitoring deck unit. The deck unit serves as playback system for recorded tows. The system includes its own winch and cable, and can be deployed from any ship having an A-frame or towing davit and 220/440 VAC power. It can operate at several knots for over 10 hours on one battery charging, and may be towed horizontally or yo-yo fashion; it can also be used vertically for profiling. Ashore, 200-frame video sequences are transferred from tape to a video disc and then fed one frame at a time into an image analysis system wherein objects may be identified, categorized, and counted. We are automating this procedure. "biological" and "physical" data are then cross-correlated and otherwise compared. The system has been to sea successfully.

FUTURE PLANS

We are just beginning to explore the image analysis problems; we will be actively developing those techniques this year. We intend to use the system to (a) describe distributions of organisms on very fine scales and (b) to use the distributions to study the development and decay of near-surface turbulent events such as Langmuir cells and convective overturn. In addition we are proposing to use the system in Austral summer 1982-83 to study the biology and physics of a "superswarm" (over 10 million tons) of adult krill (Euphausia superba) recently discovered by Shulenberger et al. in the Antarctic.

CURRENT PUBLICATIONS

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ECOLOGICAL ENERGETICS OF DEEP SCATTERING LAYER ANIMALS:
IN SITU STUDIES

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CONTRACT N00014-75-C-0152

OBJECTIVES

The overall objective of the proposed research is to examine the questions of how and why deep scattering layer animals undergo diurnal vertical migrations using an ecological energetics approach. The immediate sub-objectives are: (a) to determine in situ metabolic rates of dominant migrating and non-migrating DSL animals at depths of maximum concentration, and (b) to determine the day and night behavior of the dominant DSL animals by direct observations.

ABSTRACT

Net sampling and submersible observations have characterized the unique assemblage of animals that comprise the deep scattering layer but the functional questions of how and why many of these organisms undergo diurnal vertical migrations, in some cases as much as 800-1000 m, remain unanswered. Knowledge of the metabolic energy required by these animals and the overall energetic gains accrued, if any, for making such migrations is of prime importance in answering such questions.

We have measured the in situ metabolism of two dominant DSL animals, the gonostomatid fish Cyclothone acclinidens and the physonect siphonophore Nanomia bijuga, in the Catalina Basin off southern California, using an Alvin-operated slurp gun respirometer. Oxygen consumption rates of C. acclinidens, measured continuously over a 28-h period at the depth of capture, revealed a daily pattern. Nocturnal rates of respiration were 3-5 times higher than diurnal rates, suggesting a daily rhythm in this non-migrating species. Zeitgeber, such as tides, light and food, may serve to phase-adjust the observed respiration pattern.

Another dominant member of the DSL off southern California is the siphonophore Nanomia bijuga. In situ metabolic rates of N. bijuga were comparable to rates measured on the same species collected in warmer surface waters if a Q_{10} of 2 is employed for temperature compensation. No diurnal pattern in activity was found in N. bijuga, a confirmed diurnal vertical migrator.

Nocturnal and diurnal behavioral observations of these and other dominant DSL animals were made from DSV Sea Cliff in the Catalina Basin at depths between 300 and 1000 m in July 1980. Siphonophores were active at all times, while the myctophids, including Stenobrachius leucopsaurus and Triphoturus mexicanus, were active only nocturnally.

PLANS FOR FUTURE

(a) Continue in situ measurements of dominant DSL animals at depths of maximum concentrations using DSRV Alvin (March 1982); (b) continue in situ observations on day and night behavior of dominant DSL animals, relating them to metabolic measurements.

CURRENT REPORTS AND PUBLICATIONS

(a) K. L. Smith, Jr. and M. B. Laver (1981), "Respiration of the bathypelagic fish Cyclothone acclinidens." Mar. Biol. 61, 261-266

SOURCES AND DISTRIBUTIONS OF OCEANIC BIOLUMINESCENCE

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CONTRACT N00014-76-C-0226

OBJECTIVES

(a) To identify the organisms responsible for near-surface bioluminescence in oceanic waters, (b) to assess their relative importance in nocturnal bioluminescence production, (c) to measure the vertical and horizontal patterns of near surface bioluminescence, (d) to measure the color and intensity of the bioluminescence.

ABSTRACT

On two previous cruises to the Sargasso Sea, EN049 and EN054, we determined vertical profiles of bioluminescence in the upper 200m using a pump-through (4 liters min^{-1}) bathyphotometer. Organisms passing through the photometer were collected using 25 μm porosity nets at selected depths in the profile. Preliminary analysis of the data suggests that crustacea (mostly larvaceans and ostracods) rather than dinoflagellates produce more than half of the bioluminescence in oceanic waters.

There is considerable structure in the vertical profiles. Generally the bioluminescence maxima are not at the same depths as the chlorophyll maxima. Total bioluminescence was higher in the northern part of the Sargasso Sea than in the southern region. In addition to bathyphotometer studies, we determined the presence or absence and intensity of bioluminescence in crustacea isolated from net tows.

PLANS FOR FUTURE

(a) To study the contribution to surface displays of large crustaceans and other invertebrates too large or agile to be captured by the pump-through bathyphotometer, (b) to measure the color of the bioluminescence of surface organisms, (c) to develop methods for measuring the color of weak or intermittent bioluminescent displays, (d) to determine the organisms responsible for bioluminescent displays in temperate and boreal waters, (e) to examine and to correlate with hydrographic factors the vertical and horizontal patterns of bioluminescence.

CURRENT PUBLICATIONS

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Pelagic Molluscs and Hyperiid Amphipods of Gulf Stream
Cold Core Rings

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OBJECTIVES

(a) To continue the identification of euthecosomatous pteropods (shelled molluscs), cephalopods and hyperiid amphipods collected in Gulf Stream cold core rings, the Sargasso Sea and the Slope Water Region, (b) to compare patterns of diel migrations of these organisms occurring in these environments and (c) to compare temporal and spatial variations in these faunal components to hydrographic variation.

ABSTRACT

Vertically stratified zooplankton tows to as deep as 1600 meters have been taken on six cruises using a 1m² multiple open-closing net system. These tows were taken over a two year period and represent five cold core rings. Two of these rings have been sampled at two points each in their lifetimes - Ring D at age five and nine months and Ring Bob at age one and five months. The pteropods, cephalopods and hyperiid amphipods from these samples (as well as the pteropods and cephalopods from a larger 10m² net system) have been sorted and identified from a majority of the cruises. Changes in species composition, abundance, depth distributions and diel migrations are being examined and correlated with biological and physicochemical parameters.

Eighty tows have been completed for pteropods and cephalopods and 34 tows have been completed for amphipods. Correspondence analysis and canonical correlations on the hyperiid amphipod data are completed using the integrated water column values. The results show: 1) in Ring D the amphipod assemblage associated with the ring was distinct from that of the Sargasso Sea. The Slope Water amphipod assemblage at these two times was distinct from both the Sargasso Sea and Ring D. The ring assemblage at nine months was closer to that of the Sargasso Sea than was the ring assemblage at six months; 2) the amphipod assemblage of Ring Bob at the time of formation was indistinguishable from the Slope Water assemblage. Four months later the ring assemblage was distinct from the Slope assemblage four months later and at the time of formation; 3) the Sargasso Sea tows group tightly together regardless of month or year. This implies that the amphipod assemblage of the Sargasso Sea is very stable in both abundance and species composition. This last observation also applies to the pteropod assemblage in the Sargasso Sea - while rank order of abundance is very constant and species "agree" (high concordance values) as to which tows have highest abundance, there is no obvious correlation with season or year.

We have completed the lab analysis of all tows. Computer files are edited and being formatted for technical reports. Statistical analyses and plotting are completed. The last of several papers is in manuscript form.

PLANS FOR THE FUTURE

- (a) To prepare a joint paper which examines the overall responses of the many taxonomic groups worked up by the various investigators.

CURRENT REPORTS AND PUBLICATIONS

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- (b) Wormuth, J.H. (in press). Vertical distribution and diel migration of Euthecosomata in the Northwest Sargasso Sea. Deep-Sea Research, 28A:
- (c) Greenbelt, P.W., E. Shulengerger and J.H. Wormuth (in press). Fine scale spatial pattern in zooplankton distributions: a comparison of multiple net systems vs. doppler shift acoustic sampling. Deep-Sea Research.
- (d) Hart, A.D. 1981. Pelagic Amphipods of the Gulf Stream Cyclonic Cold-Core Rings. Ph.D. Dissertation, Texas A&M University. 210 pp.

BIODETERIORATION

II. MECHANISMS GOVERNING INDUCTION AND INHIBITION OF SETTLING AND ESTABLISHMENT IN SOME SESSILE AND BENTHIC MARINE ORGANISMS

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OBJECTIVES

The over all project objective is to investigate endocrine and other biochemical systems governing induction and inhibition of settling, growth and establishment in various crustacea and seaweeds. 1980-1981 objectives: 1) To begin studying, under culture conditions, physiological factors involved in the crustacean molt cycle. 2) To measure glycogen deposition relative to constant and cyclic temperature regimes in megalopa larvae of the mud crab Rithropanopeus harrissi.

ABSTRACT

Work centering on glycogenesis and use of glycogen energy reserves by Rithropanopeus harrissi demonstrated that larvae reared at constant temperature (32.5 degrees C) and on metamorphosis to the megalopa stage exposed to cyclic temperatures (30 to 35 degrees C) were not able to adapt adequately to maintain glycogen levels characteristic of control megalopa larvae. Early-stage larvae maintained in an environment with regularly fluctuating temperatures until the molt to megalopa fared somewhat better in maintaining glycogen levels, although over all glycogen levels were depressed by comparison with those levels in test animals maintained at constant (32.5 degree C) temperature throughout the experimental periods.

Information obtained during this study indicates that physiological response to cyclic versus constant temperatures is at least to some extent reflected in glycogen level. Because part of the glycogen used by crab larvae functions as a precursor of chitin, glycogen demand is high during and shortly after molting. It was thought that changes in glycogen levels might well be reflected in interrupted molting, but this was not observed in this study. Although data indicated significant differences in measured levels between some test and control crab larvae, differences were not great and megalopa larvae metamorphosed to the first crab stage after the usual 48-52 hours.

PLANS FOR THE FUTURE

(a) immuno-cytochemical studies of parasitic red algae spores and host species will be initiated for the purpose of isolating and identifying substances involved in induction or inhibition of germination.

(b) barnacles (Amphitrite and Lepas) will be established in culture and Menippe adults will be maintained in order to begin radioimmunoassay of gonadotrophin effects on moulting.

MICROBIAL FOULING AND ITS EFFECT ON
POWER GENERATION

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WORK UNIT NO. NR

104-162

CONTRACT N 00014-80-C-0475

OBJECTIVES

(a) Develop a unified theory which describes biofilm development and the resulting energy losses in heat exchange equipment, (b) investigate the effect of particulate inclusions and inorganic constituents within the biofilm on biofilm structure and energy losses, (c) develop methods for measuring the extent of biofouling, its effect on energy losses, and the effectiveness of biofilm destruction processes, (d) determine the effectiveness of selected chemical techniques for biofouling control.

ABSTRACT

Biofilms develop on the tube side (i.e., cooling water side) of power plant condenser surfaces increasing fluid frictional resistance and heat transfer resistance. Biofilm accumulation leads to increased steam pressure on the shell side (i.e., the steam side) of the condenser and reduces turbine efficiency. Consequently, more fuel is consumed per unit power produced. Increases in fluid frictional resistance also lead to increased power consumption for pumping. The resulting effects of biofouling in ship systems is increased energy consumption for normal operation.

This project was initiated on 15 May 1980. Since that time, we have accomplished the following: (1) structured mathematical models have been developed to describe biofilm growth and the effect of biofilm on heat transfer resistance, (2) two different experimental systems have been constructed and are being used to study biofilm development and interaction of biofilm with inorganic constituents within the biofilm matrix, (3) analytical techniques have been developed for our continuing work with defined and mixed bacterial cultures, (4) biofilm development with S. natans and Ps. aeruginosa has been studied and compared with particular attention to biofilm morphology and the effect on frictional resistance, and (5) initial experiments on rates of scaling and its influence on heat transfer resistance have been completed.

PLANS FOR THE FUTURE

(a) Modify and enhance the biofouling computer models, as necessary, to obtain consistency with experimental results, (b) study the development of marine biofilms and their effect on frictional and heat transfer resistances, (c) compare heat transfer resistance of biofilm to that of scale and biofilm-scale associations, and (d) determine the biofouling control effectiveness of chl line and cathelco in titanium and copper-nickel alloys.

CURRENT REPORTS AND PUBLICATIONS

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- (b) Picologlou, B.F., Zilver, N. and Characklis, W.G. "Effect of Biofilm Growth on Hydraulic Performance," J. of Hydraulics Div., ASCE, 106, HY5, 733-746 (1980).
- (c) Bryers, J.D. and Characklis, W.G. "Initial Biofouling in a Turbulent Flow System: Overall Kinetics," Water Research, 15, pp. 483-491 (1981).
- (d) Characklis, W.G. "Fouling Biofilm Development: A Process Analysis," accepted by Biotechnology and Bioengineering.
- (e) Characklis, W.G., Nimmons, M.J. and Picologlou, B.F. "Influence of Fouling Biofilm on Heat Transfer," accepted by Heat Transfer Engineering.
- (f) Trulear, M.G. and Characklis, W.G. "Dynamics of Biofilm Processes," accepted by Journal Water Pollution Control Federation.
- (g) Characklis, W.G., Bryers, J.D., Trulear, M.G. and Zilver, N. "Dynamics of Biofilm Processes: Methods," accepted by Water Research.
- (h) Characklis, W.G., Zilver, N. and Turakhia, M., "Fouling and Heat Transfer" in Fouling in Heat Exchange Equipment (Chenoweth & Impagliazzo, eds.), ASME, HTD, Vol. 17, pp. 1-16 (1981).
- (i) Characklis, W.G., Bryers, J.D., Trulear, M.G., Zilver, N. "Biofouling Film Development and Its Effects on Energy Losses: A Laboratory Study," Chap. 5, pp. 49-76 in Condenser Biofouling Control, Garey, J.F. (ed), Ann Arbor Science, Ann Arbor, MI (1980).
- (j) Bryers, J.D. and Characklis, W.G. "Measurement of Primary Biofilm Formation," Chap. 11, pp. 169-183 in Condenser Biofouling Control, Garey, J.F. (ed), Ann Arbor Science, Ann Arbor, MI (1980).
- (k) Characklis, W.G., Trulear, M.G., Stathopoulos, N. and Chang, L.C., "Oxidation and Destruction of Microbial Films," Chap. 32, pp. 349-368 in Water Chlorination: Environmental Impact and Health Effects, Vol. 3, Jolley, R.L. (ed), Ann Arbor Science, Ann Arbor, MI (1980).
- (l) Characklis, W.G., "Process Analysis in Microbial Systems: Biofilms as a Case Study," in Mathematical Techniques in Microbiology (Bazin, ed), Academic Press, London, in press.
- (m) Characklis, W.G., Zilver, N. and Turakhia, M. "Microbial Films and Energy Losses," in Marine Biodeterioration (Costlow and Tipper, eds), U.S. Naval Institute Annapolis, MD (1981).
- (n) Characklis, W.G. "Microbial Fouling: A Process Analysis," in Fouling of Heat Transfer Equipment (Somerscales and Knudsen, eds.), pp. 251-291.
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The above list represents references to reports and publications prepared or modified since July 1, 1980.

A PHYSIOLOGICAL INVESTIGATION OF THE
ADHESION OF DIATOMS TO SURFACES

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WORK UNIT NO. NR 083-060

CONTRACT N00014-80-C-0042

OBJECTIVES

(a) To investigate the means by which fouling diatoms attach to submerged surfaces. (b) to examine the chronology of the events (c) to analyze chemically any attachment polymer (adhesive) produced by the diatom. (d) to examine the involvement of divalent cations.

ABSTRACT

The diatom Amphora coffeaeformis is commonly found on surfaces treated with toxic antifoulant preparations. We have used this organism in an attachment assay and are now investigating the rate at which it becomes firmly attached to glass and polystyrene surfaces. There appear to be no differences visible using the scanning electron microscope (SEM) between cells attached in five minutes and cells attached for eighty minutes. Small projections ('fingers') of polymer are exuded from the raphe canal. During the attachment process these end in adhesion foci at the substratum. When these adhesive projections are broken mechanically during processing for S. E. M.- examination, they appear to shrink slightly in the plane of the projection. This may indicate that these organelles are contractile in nature. When cells are detached from a glass surface using the calcium - chelator EGTA, the diatom attachment substance is left behind in the pattern of the raphe canals.. Preliminary analyses of this material showed that it is not a protein. Further analyses are planned concerning its chemical nature.

Diatoms need 2.0 mM calcium to attach to surfaces. They are motile under these circumstances, so the adhesion is correctively classified as a 'temporary' and probably involves a Stefan-type adhesive. Diatoms also adhere in the presence of strontium ions. The adhesion appears to be qualitatively as strong as with calcium, but the cells are not motile. When these attached cells are examined in the S.E.M., structural differences in the means of attachment are apparent. The adhesive coming from the raphe does not appear as relatively uniform projections, but is a membranous reticulate structure. Further investigation of this phenomenon may shed light on the actual role of calcium in the attachment process, as well as its involvement in diatom motility.

Previously we have reported on the effect of various drugs and poisons on diatom attachment. On the basis of their effects on other organisms unrelated to diatoms, we postulated the mode of action of these compounds on diatoms was to interfere with calcium homeostasis. To confirm these ideas it is necessary to see how these drugs, etc., do interfere with the ability of the diatom cell to control its internal calcium concentration. To this end we currently have under development an assay for calcium uptake by diatom cells, using ^{45}Ca . Because of the high extracellular concentrations necessary for attachment and low internal concentration changes anticipated, we have encountered considerable experimental difficulties, especially with regard to background measurements.

FUTURE PLANS

When the ^{45}Ca -transport assay is found to be satisfactory we will investigate, in particular, the role of cycloheximide in preventing diatom attachment. We will also continue studies on the analysis of the attachment polymer and the structurally-different types of adhesive promoted by calcium and strontium. We expect this research to provide new directions in the search for chemical antifoulant compounds.

CURRENT REPORTS AND PAPERS

The attachment of microfouling diatoms. Barbara Cooksey, Keith E. Cooksey, Christine Miller, John H. Paul, Robert W. Rubin and Daniel Webster. Office of Naval Research Symposium on Marine Biodeterioration, Bethesda, Md. April, 1981.

Requirement for calcium in adhesion of a fouling diatom to glass. Keith E. Cooksey. Appl. Env. Microbiol. 41:1378-1382, 1981.

STUDIES ON MOLTING AND GROWTH IN LARVAL AND ADULT BARNACLES AND LARVAL DECAPODS

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CONTRACT NO0014-78-C-0294

OBJECTIVES

(a) To demonstrate the existence of naturally occurring chemicals that attract and repel settling barnacles, (b) to examine cyprid responses to these chemicals, (c) to characterize, isolate, and identify the chemicals involved, (d) to investigate the control of gametogenesis by environmental cues as mediated by hormonal factors, (e) to examine several morphological changes which occur during metamorphosis, (f) to investigate the regulation of molting, metamorphosis and regeneration by hormonal factors, and (g) to define the effect of growth on passage through the molt cycle and the timing of ecdysis to the cyprid larva.

ABSTRACT

In the past year work has focused on chemicals in the soft gorgonian coral Leptogorgia virgulata that enhance and inhibit barnacle settlement. Settlement tests have been accomplished on neutral or inactive substrata that have been treated with attractants and repellents or toxins. Settlement of Balanus improvisus and B. amphitrite is significantly inhibited in water treated with homogenized gorgonian soft tissue and in water previously exposed to living gorgonians. These data suggest chemical repellency or toxicity. The effect occurs at homogenate concentrations of 0.1 g wet weight per liter, but not at 0.01 g/l. The repellent/toxin is not a by-product of bacterial degradation. It appears to be soluble in seawater, rather than adsorbed to surfaces, and is dialyzable. A settlement attractant of high molecular weight is also present in the soft tissue, and in the gorgonian axial skeleton. The sea pansy, Renilla reniformis, has a repellent/toxin to barnacle settlement that is even more powerful than that of Leptogorgia.

In addition, the annual reproductive cycle of two local barnacles, Balanus amphitrite Darwin, 1854 and Balanus subalbidus Henry, 1973 has been studied. In the former species, more than 50% of each monthly sample showed ovarian and testicular ripeness throughout the year. Meanwhile, the latter species showed quiescence of both male and female reproductive systems from December through March. In both species peak brooding activity is in the summer months while less than 5% of each winter sample carry broods. Central nervous systems of both species, fixed and stained for Paraldehyde Fuchsin positive cells, showed no difference in staining properties in summer versus winter samples. Although reproductive activity in these barnacle species, especially B. subalbidus, shows some correlation with photoperiod and water temperature in the field, we have not been able to correlate gametogenesis or environmental factors with stainable changes in neurosecretory activity within the central nervous system of either species.

To assess the potential for direct hormonal control of reproduction, individuals of Balanus amphitrite which were reproductively quiescent, were injected with three different doses of estradiol-17B — 0.02, 0.2, and 2.0 ng per animal — at weekly intervals for one month. Both the 0.2 and 2.0 ng doses caused a significant increase ($P < 0.01$). 10% and 30% respectively, in the diameters of mature oocytes. This experiment demonstrated the

possibility that steroids may be involved in the chain of physiological factors that translate environmental cues into the onset of gametogenesis within barnacles.

The final phase of work on growth and molting in Balanus eburneus larvae consisted of examining the relationship between the rate of energy reserve accumulation and molting rate in sixth instar nauplii. Energy reserves are sequestered in specialized lipid storage cells ("oil cells") which are attached to the outer surface of the midgut of the nauplius. The energy reserve level present within a nauplius at a given point in time is estimated by determining the total volume of these oil cells. Past work had suggested that the duration of the sixth naupliar instar was inversely proportional to the rate of increase in oil cell volume. The results of the present work on the relationship between oil cell volume and instar duration is as follows. Instar duration is inversely proportional to the rate of oil cell volume increase in nauplii which begin the sixth instar with a low oil cell volume. However, instar duration is largely independent of the rate of oil cell volume increase in nauplii which begin the sixth instar with moderate to large oil cell reserves. Thus the rate of passage through the instar is not dependent upon oil cell accumulation per se, but upon the existence and maintenance of a small (ca. $1.5 \times 10^4 \text{ mM}^3$) oil cell volume throughout the instar.

PLANS FOR THE FUTURE

(a) To continue to investigate the effects of natural attractants and repellents on cyprid behavior, (b) to determine further the chemical and physical characteristics of these settlement factors, (c) to identify them, (d) to evaluate their use in the control of biofouling, (e) to test the effect of central nervous system extracts from reproductively active animals on ovarian development within reproductively quiescent animals, (f) to test the effect of Ecdysterone *in vivo* on mature oocytes and on the activity of the cement glands, (g) to determine in the laboratory to what extent gametogenic quiescence in Balanus subalbidus is influenced by photoperiod versus water temperature.

CURRENT REPORTS AND PUBLICATIONS

- (a) J.A. Freeman and J.D. Costlow (1980), "Role of ecdysterone and molting-inhibiting hormone on premolt protein synthesis in barnacle epidermis." (in press)
- (b) J.A. Freeman and J.D. Costlow (1980), "Epidermal cell changes during metamorphosis in Balanus amphitrite cyprids." (in preparation)
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The following list of papers is currently in preparation, by J.A. Freeman and J.D. Costlow:

1. The cyprid molt cycle of the barnacle, Balanus amphitrite.
2. Endocrine regulation of premolt in Balanus amphitrite cyprids.
3. Endocrine control of spine epidermal resorption during metamorphosis in Rhithropanopeus harrisi.
4. Post-metamorphosis molt cycle regulation in juveniles of the crab, Rhithropanopeus harrisi.
5. Spine regeneration in larvae of the crab, Rhithropanopeus harrisi.
6. Crustacean growth and metamorphosis interaction of endocrines and the epidermis (to be finished in January 1982).

THE EFFECTS OF ORGANIC MATTER AND SURFACE ASSOCIATED MICROORGANISMS ON METAL CORROSION IN THE MARINE ENVIRONMENT

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WORK UNIT NO. NR 083-300

CONTRACT N00014-76-C-0076

OBJECTIVES

The objectives over the past year have been to: (a) define the growth limitations of two highly copper-resistant bacteria, (b) determine the effect of these two organisms on copper corrosion under flow-through conditions, (c) postulate the mechanism(s) by which these bacteria resist copper and by which they influence corrosion.

ABSTRACT

The copper resistance of several representative periphytic bacteria isolated from copper bearing surfaces was evaluated. Two bacteria, a gram positive Micrococcus sp. (B-8-12) and gram negative Pseudomonas sp. (B-3) were found to be resistant to copper levels up to 75 and 100 ppm, respectively. Because of their ability to: (1) grow in the presence of high levels of copper, and (2) grow on either glutamic acid or glucose as sole carbon sources, these organisms were chosen for subsequent flow-through corrosion experiments. The effects of carbon source, ammonium and phosphate levels on the growth of these organisms in copper-free and copper-containing media are being determined.

As a prelude to the inoculation of the flow-through corrosion cells with the bacteria, copper was subjected in the cells to sterile seawater for 60 days then to sterile seawater plus nutrients for additional 60 days. Corrosion rate measurements were taken throughout these periods to provide us with internal baselines, and to confirm the corrosion behavior of copper as a function of time. Furthermore, additional copper specimens were placed in the flow-cell systems and removed before the changeover was made between seawater and seawater plus nutrients. Scanning electron microscopy of these specimens revealed uniform corrosion patterns, and X-ray diffraction analysis indicated the presence of cuprite. Similar analyses will be made prior to inoculation.

The growth of Pseudomonas sp. (B-3) appeared to be stimulated by the presence of copper with either glucose or glutamic acid as sole carbon sources. In the case of glutamic acid, and copper concentrations higher than 10 ppm, this organism produced a red biomass under microaerophilic conditions but not when vigorously aerated. It was found that the bacteria produced internal red granules which fluoresce yellow when excited with green light. These granules are being purified for further analysis.

CURRENT PUBLICATIONS

Gordon, A.S., S.M. Gerchakov and L.R. Udey. 1981. The effect of polarization on the attachment of marine bacteria to copper and platinum surfaces. Can. J. Microbiol. 27:698-703.

Gerchakov, S.M., L.R. Udey and F. Mansfeld. 1981. An improved method for analysis of polarization resistance data. Corrosion (in press).

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BIOLOGY OF STONE AND WOOD BORERS IN MONTEREY SUBMARINE CANYON
AND DEEPER WATER OFF CENTRAL CALIFORNIA

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OBJECTIVES

(a) To determine the horizontal and vertical distribution of stone and wood boring animals in the deeper waters of Monterey Bay and the Monterey Submarine Canyon, (b) by using experimental panels of wood, stone, and concrete placed at various depths to determine settling time, rates of growth, and longevity of borers, (c) to experimentally monitor the growth of stone borers by placing them in artificial burrows in stone panels then follow growth through x-ray techniques, and (d) learn, if possible, the mechanisms used by bivalves in boring into stone.

ABSTRACT

During this past year we have extended our studies into deeper water in the Monterey Submarine Canyon and have deployed recoverable arrays at depths of 500 and 650 m. At each of these depths arrays containing a variety of wood panels plus shale and concrete were placed and recovered at 3 and 6 month intervals. Some of the experimental materials were placed in contact with the substrate, others were placed on the array several meters above the bottom. Acoustic releases were employed and proved more reliable than the timed releases used in former years. The only species of borer found at both 500 and 650 m depth was the wood boring pholad Xylophaga washingtona. Panels of pine, douglas fir, redwood, and white oak were infested with enormous numbers of small Xylophaga after 3 months exposure, and after 6 months most of the wooden panels were totally destroyed by these borers. The intensity of infestation and wood destruction was comparable at the two depths. Panels became equally infested regardless of the date or season of initial deployment. Wooden panels arrayed some distance above the bottom and separated from other wood lower down were either not infested at all or carried very few borers. This finding indicates that the larvae of Xylophaga search for wood at the water-sediment interface, perhaps moving about on the bottom.

No stone or concrete borers were found at either depth studied. Many juvenile Xylophaga settled in depressions on the stone samples, but were unable to penetrate the substrate and died, leaving the shell valves adhering to the stone.

Our studies on growth rates using radiographic techniques have continued and we now have several series of x-ray photographs of borers in artificial burrows in stone. In several of these series we have bimonthly photographs over a 14 month period clearly illustrating the rates of growth in stone of varying hardness. Two papers on these x-ray studies have been accepted for publication.

During the past year we have also continued our study on the resistance of various tropical woods to long-term exposure to wood borers in Monterey harbor.

PLANS FOR THE FUTURE

During the coming year (a) additional arrays of wood and stone panels will be deployed in greater depths than 600m and these will be recovered after 3 and 6 month intervals for analysis, (b) the monitoring of growth rates using x-ray techniques will continue, and (c) a cooperative study with scientists at the University of Miami and the Woods Hole Oceanographic Institute will be initiated to determine the methods used by bivalve stone borers in penetrating hard substrates.

CURRENT PUBLICATIONS

(a) R. Morris, D. P. Abbott & E. C. Haderlie (1980), "Intertidal Invertebrates of California." Stanford University Press, November 1980. (Book of nearly 1,000 pages)

(b) E. C. Haderlie & R. C. Tipper (1980), "5th International Congress on Marine Corrosion and Fouling." ONR London Conference Report C-8-80.

(c) E. C. Haderlie (1981), "Influence of terminal end of burrow on callum formation in the rock boring clam Penitella penita (Conrad, 1837) (Bivalvia: Pholadidae)." The Veliger 24 (1): 51-53.

(d) E. C. Haderlie (1981), "Growth rates of Penitella penita (Conrad, 1837), Chaceia ovoidea (Gould, 1851) (Bivalvia: Pholadidae) and other rock boring marine bivalves in Monterey Bay." The Veliger (in press)

(e) E. C. Haderlie (1981), "Monitoring growth rates in wood and rock boring marine bivalves using radiographic techniques." Proceedings of the 5th International Biodeterioration Symposium, Aberdeen. (Accepted for publication)

BIOENERGETICS OF WOOD BORING MOLLUSCS

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WORK UNIT NO. NR 083-004

CONTRACT N00014-79-C-0071

OBJECTIVES

Our research is intended to describe the relationship of storage metabolism, reproductive activity and nutritional status in wood boring molluscs. This is being effected through a comparative study of the energy budgets and gross biochemical compositional changes with growth in an oviparous teredinid Bankia gouldi, a larviparous teredinid Teredo navalis, and an oviparous pholad Martesia cuneiformis. This comparison will (i) provide a description of the relative quantities and fates of energy derived from ingested wood and phytoplankton in the teredinid species under investigation, (ii) provide physiological rate measurements to support previous evidence of the importance of wood boring marine bivalves in nutrient cycling in the oceans, and (iii) through comprehensive description of the biochemical and physiological changes occurring during metamorphosis, growth and reproduction (especially those related to wood utilization and carbohydrate metabolism) suggest specific processes and/or biochemical pathways which should be targeted for inhibition as part of a marine biofouling control program.

ABSTRACT

Large scale (50-200 L culture volume) rearing of the larval forms of Teredo navalis, Bankia gouldi and Martesia cuneiformis for growth and biochemical composition studies, and the provision of further broodstock and post metamorphosis individuals for physiological studies has continued during 1980. Comparative test of the Tahitian strain of the flagellate Isochrysis galbana (TISO) as a food species with a previously used diet of a Thalassiosira pseudonana - Isochrysis galbana mixture demonstrated that TISO was a superior food both in terms of growth and ease of culture as a food species. On the newly adopted diet at a temperature of 23°C, T. navalis, B. gouldi and M. cuneiformis reach metamorphosis in 18-22, 23-27 and 24-30 days respectively. Measurements of larval respiration rate have been made: those of T. navalis and B. gouldi larvae are similar to one another (2.0-0.5 $\mu\text{O}_2/\text{mg}$ dry weight/hour at straight hinge and pediveliger stage respectively) but higher than those recorded for M. cuneiformis straight hinge and pediveliger stages (0.1-0.06 $\mu\text{O}_2/\text{mg}$ dry weight/hour, respectively).

Growth studies of post metamorphosis T. navalis and B. gouldi in individual fir dowels have continued during 1981. Preliminary analysis of data indicates no enhancement of either growth rate or maximum size attained over a six month period in a comparison of specimens whose diet was supplemented by the continuous addition of a monoculture of TISO with specimens maintained in wood held in one micron filtered sea water. Gross biochemical analysis of individuals grown in the filtered water regime showed a rapid increase in carbohydrate content in both T. navalis and B. gouldi following metamorphosis. Mean carbohydrate contents of 22.9% and 22.8% percent of dry weight respectively were recorded for animals in excess of 5 mg dry tissue weight (approximately two weeks after metamorphosis) clearly indicating the heavy reliance on carbohydrate as an energy reserve in the species.

Further development and use of the multiparameter physiological recording apparatus described in the 1980 abstract has been effected in 1981. We have conclusively demonstrated that both T. navalis and B. gouldi can effect boring and filtration simultaneously over extended periods of time (days). Respiration is tightly coupled to boring rather than filtering activity. Faecal pellets are produced continuously during boring and for up to 60 minutes after cessation of boring. A short movie has been made describing the recording apparatus and illustrating the correlation of boring activity and recording of adductor action potentials (electromyograms).

Marine bivalves are generally ammonotelic; however, in earlier work we were unable to demonstrate ammonia and amino acid excretion in T. navalis and B. gouldi. Spiking experiments showed that both species are capable of uptake of both substrates in the dissolved form. This phenomenon was further investigated in a collaborative effort with Dr. Robert D. Prusch of the Marine Biological Laboratory at Woods Hole. Carrier mediated active transport of ^{14}C -alanine was demonstrated for both the pediveliger larvae and adult forms of Lyrodus pedicellatus. Net influx of alanine was confirmed by the flourescamine technique. A comparison of uptake in individuals removed from the wood with ligated siphons versus control non-ligated specimens showed the site of uptake to be in the mantle cavity rather than via the external mantle epithelium. Recent work by J. Waterbury at Woods Hole Oceanographic Institution with nitrogen fixing, microaerophilic, cellulase producing bacteria isolated from the Gland of Deshayes in T. navalis, B. gouldi and L. pedicellatus suggests that uptake via these extracellular bacterial associates in the gills requires further investigation.

FUTURE PLANS

Completion of studies on (a) mass culture and larval physiology of M. cuneiformis, (b) post metamorphosis growth, physiology and biochemical composition in M. cuneiformis, (c) post metamorphosis physiology of T. navalis and B. gouldi, (d) compilation of data and construction of an energy budget for the different life stages of each species.

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(b) S. M. Gallagher and R. Mann (1981), Use of lipid specific staining techniques for assaying condition in cultured bivalve larvae. J. Shellfish Res. 1(1) (in press).

(c) R. Mann (1981), Nutrition in the Teredinidae. In: Symposium on Marine Biodeterioration. U.S. Office of Naval Research (in press).

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(e) R. Mann (1981), Shipworm nutrition: a review of recent work with an unusual bivalve. Nat. Shellfish Ass. 1981 A.G.M. (abstract).

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(h) A. E. Wright, C. M. Cavanaugh, S. M. Gallagher, R. Mann and R. D. Turner (1981), Preliminary observations of bacteria and shipworms. Bio. Bull. (abstract) in press.

(i) R. Mann (1981), Control of reproduction in marine bivalve molluscs: endogenous and exogenous factors. Intern. Soc. Invert. Reprod. A.G.M. (abstract) in press.

THE ROLE OF MICROORGANISMS IN
MARINE FOULING AND BORING PROCESSES

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CONTRACT N00014-76-C-0042

OBJECTIVES

(a) To identify the mechanisms by which microorganisms induce settlement of the fouling polychaete, Janua (Dexiospira) brasiliensis. (b) To describe the processes by which J. brasiliensis larvae recognize the settlement cues produced by bacterial films. (c) To determine whether biochemical cues produced by bacteria induce the settlement of other invertebrate fouling organisms and algal zoospores. (d) To complete our studies on interactions between microorganisms and the crustacean and molluscan wood-borers.

ABSTRACT

Our research over the past several years has focused on interactions that occur between microorganisms and marine boring and fouling invertebrates. During the past two years we have studied the role that microorganisms play in the settlement of marine invertebrate larvae.

During the past year we have carried out extensive studies on the role of surface films of bacteria in the settlement and metamorphosis of invertebrate larvae. We have shown that certain bacteria present in microbial surface-films produce biochemical cues that induce the settlement of Janua (Dexiospira) brasiliensis larvae. Biochemical cues produced by bacteria may induce the settlement of other fouling invertebrates and algae as well.

During the past year, we have demonstrated that larvae of J. brasiliensis settle and metamorphose on films of specific bacterial strains as well as multi-species microbial films (Kirchman et al., 1981). Our results suggest that Janua settlement is induced by the quality and/or quantity of extracellular polymers produced by selected bacterial species in natural, complex primary films.

Our research has demonstrated that larvae of J. brasiliensis settle and metamorphose on bacterial films. Larvae fail to settle on slides coated with the diatom Nitzschia or gum arabic or on clean slides. Single-species bacterial films are not equally effective in inducing larval settlement. Some bacterial strains failed to induce settlement altogether, while others induced metamorphosis at rates of up to 90%.

The active component, from the bacterial film, which induces larval settlement remains to be identified. Our data suggest that the active biochemical cue is bound to the microbial film. Antibiotics did not inhibit larval settlement and adults were seldom seen on the clean control slide adjacent to the microbially filmed slide, or elsewhere in the culture dish. Formalin-killed films successfully induced metamorphosis.

In addition, preliminary experiments run in our laboratory suggest that

bacteria may be involved also in the settlement of bryozoan larvae. This research is being conducted jointly with Prof. Robert Woollacott of Harvard University.

Our findings concerning the involvement of bacteria in the settlement of Janua larvae, and possibly in the settlement of bryozoan larvae, suggest that bacteria may play similarly important roles in the attachment of a range of other invertebrate fouling organisms. During the next year we plan to test our model for the induction of larval settlement by bacteria with other important fouling invertebrates.

Algal fouling of ship hulls and marine equipment is a world wide problem of enormous proportions. Little is known about the initial attachment of algal zoospores. It is possible that algal zoospores recognize appropriate surfaces for attachment by a biochemical mechanism similar to that which we are studying in the settling larvae of marine invertebrates. Algal zoospores may detect bacterially produced biochemical cues on surfaces, which may induce their settlement.

Our research during the past several years on marine wood-borers is nearing completion. The results from this research concerning the interactions that occur between microorganisms and the crustacean wood-borer, Limnoria, suggest several new control methods.

PLANS FOR FUTURE

(a) Continuation of our studies on the induction of larval settlement by biochemical cues produced by bacteria in marine biofilms. (b) Determination of the role that bacteria play, in general, in settlement of marine fouling invertebrates and algae. (c) Study of other critical interactions that occur between microorganisms and marine fouling organisms. (d) Completion of our studies on the interactions between microorganisms and crustacean wood-borers.

CURRENT REPORTS AND PUBLICATIONS

(a) P.J. Boyle and R. Mitchell (1981), "The external microflora of a marine wood-boring isopod." *Applied and Environ. Microbiol.* (In press: to be published Oct. 1981).

(b) P.J. Boyle and R. Mitchell, "Bacteria associated with the digestive tract of the deep-sea isopod Bathynomus giganteus." (Submitted to *Current Microbiol.*).

(c) P.J. Boyle and R. Mitchell, "Vibrio cholerae associated with a tropical crustacean wood-borer." (In Prep.).

(d) S. Graham, D. Kirchman and R. Mitchell (1980), "Larval settlement on microbial films: A model system." *Biol. Bull.* 159, 460.

(e) D. Kirchman, L. Mazzella, R. Mitchell and R. Alberte (1980), "Bacteria on Zostera surfaces." *Biol. Bull.* 159, 461.

(f) D. Kirchman, S. Graham, D. Reish and R. Mitchell, "Larval settlement and metamorphosis of Janua (Dexiospira) brasiliensis (Grube) (Polychaeta: Spirorbidae) is mediated by lectins." (Submitted to *Science*).

(g) D. Kirchman, S. Graham, D. Reish and R. Mitchell, "Bacteria induce settlement and metamorphosis of Janua (Dexiospira) brasiliensis (Grube)." (Submitted to *J. Exp. Mar. Biol. Ecol.*)

(h) D. Kirchman and R. Mitchell, "Contribution of particle-bound bacteria to total microheterotrophic activity in natural waters." (Submitted to *Appl. and Environ. Microbiol.*)

BIOCHEMICAL CONTROL OF MARINE FOULING

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WORK UNIT NO. NR 104-160

CONTRACT N00014-80-C-0310

OBJECTIVES

Our principal objectives were: (a) to significantly accelerate our testing of the generality and significance of our major research findings, derived from experiments with molluscs, by extension of our studies to other important groups of marine fouling animals; (b) to characterize further the basic biochemical and environmental mechanisms which control the recruitment of larvae and marine fouling; (c) to investigate biochemical mechanisms, and potentially useful strategies, for the inhibition of marine fouling; and (d) to directly test the applicability of our laboratory results to the control of fouling on immersed surfaces in the ocean.

ABSTRACT

The recruitment of many fouling species to surfaces immersed in the ocean is dependent upon detection, by the planktonic larvae, of specific biochemical triggers required for induction of larval settlement, attachment, and metamorphosis. Our major research accomplishment has been the finding that these naturally required biochemical inducers of fouling, in the case of certain molluscan species, comprise a family of macromolecular conjugates of simple amino acid-derived neurotransmitter analogs, produced by algae and bacteria associated with the recruiting substrates. These findings have provided the first available model system with which it has been feasible to experimentally analyze and define the biochemical mechanisms which control the fouling sequence in marine animals, and to develop and test biochemical inhibitors of these processes which may prove useful for the control of marine fouling.

(a) We have verified the generality and significance of our principal research findings, and have validated conclusions derived from studies of the model molluscan (*Haliotis*) system, in a major acceleration of our work on the tube-building and cementing marine worms of the genus *Phragmatopoma*. Tubes of these attached animals form heavy and massive concretions which foul immersed surfaces and constitute hazards to navigation. We have found that the massive and gregarious recruitment of *Phragmatopoma* from the plankton depends upon larval detection of required biochemical inducers on the tubes of the adults. We have established that these required inducers are specific macromolecular conjugates of amino acid-derived neurotransmitter analogs, which thus control fouling by the tube-building worms by a mechanism strictly analogous to that which we previously found to control induction of the fouling sequence in a number of molluscs.

(b) In further studies with the model molluscan and tube-building worm fouling systems, we have found that induction of the fouling sequence requires direct contact and stereochemically specific recognition of inducing biochemicals. We have elucidated the stereochemical specificity requirements for induction of the fouling sequence in the model *Haliotis* molluscan larval system. These results have enabled

us to successfully "label" and characterize the epithelial chemosensory receptors upon which the planktonic larvae depend for their chemical recognition of recruiting (fouling) surfaces. Using radioactively labeled biochemical inducers of settlement and metamorphosis, we have determined that larval "competence" for induction of fouling reflects the development and biochemical activation of $\geq 10^{12}$ specialized epithelial chemosensory receptors per larva, which bind and respond to the inducing molecules. Activation of these receptors by recruiting surface biochemicals triggers the fouling sequence. These studies are the first in which chemosensory receptors controlling induction of marine fouling have been successfully labeled, identified and characterized.

We also have identified biochemical and cellular processes which act as essential transducers of the signals that trigger larval recruitment and fouling. These essential transducers include induced fluxes of chloride and calcium ions; 3', 5'-cyclic AMP; action of specific larval enzymes; and secretion of larval glycoproteins. We also have discovered a major role of certain amino acids, which can act as facilitators or promoters of marine fouling, when present in sufficient concentration in the dissolved organic material (DOM) of coastal seawater.

(c) We have developed and tested a series of new, non-toxic biochemical inhibitors which block larval settlement and induction of the fouling sequence in Haliotis. These new inhibitors (including both analogs of naturally required inducers, and lectins) act by stereochemically specific blockade of the chemosensory receptors that normally detect inducers of the fouling sequence. The effectiveness of certain of these non-toxic biochemical inhibitors is comparable to the effectiveness of conventionally used toxic heavy metals and halogenated hydrocarbons, in blocking the fouling sequence in Haliotis larvae. Non-toxic agents of broader species-specificity now are under investigation and development.

(d) We have accelerated the direct testing of the applicability of our laboratory findings to the control of marine fouling on immersed surfaces in the ocean. Preliminary tests of biochemical inhibitors of fouling, in comparisons with conventional organotin and copper antifouling paints, have been started with cooperation and assistance from representatives of U.S. industry.

PLANS FOR THE FUTURE

(a) Further characterization of biochemical inducers, chemosensory receptors, and essential transducers which control induction of the fouling sequence in tube-building worms and Haliotid molluscs; (b) design and testing of new inhibitors of marine fouling based upon these findings, concentrating on inhibitors of the essential larval transducers of fouling-signals; (c) increased direct ocean testing of biochemical control of marine fouling; and (d) extension of these studies to important marine fouling organisms (mussels, etc.) which attach to submerged surfaces with strong byssus fibers.

CURRENT REPORTS AND PUBLICATIONS

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(b) D. E. Morse, N. Hooker and H. Duncan (1980), "GABA induces metamorphosis in Haliotis, V: stereochemical specificity." Brain Res. Bul. 5 (S2), 381-387.

(c) D. E. Morse (1981), "Biochemical control of larval recruitment and marine fouling." Proc. Symp. Marine Biodeterioration, ONR (in press).

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PHYSICAL MEASUREMENTS OF WAVE-GENERATED FORCES ON A BIOLOGICALLY
SIGNIFICANT SCALE, AND THEIR ECOLOGICAL CONSEQUENCES

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WORK UNIT NO. NR 083-498X

CONTRACT N00014-79-C-0611

OBJECTIVES

(a) To design and construct a telemetry system and recording dynamometers to measure the hydrodynamic forces imposed on intertidal fouling organisms by breaking waves, (b) to use these instruments to characterize the water flow during breaking waves and to measure the wave forces imposed on a variety of animals attached to rocky shores, (c) to use these data to examine the mechanical design and adhesive capabilities of fouling organisms, and (d) to re-examine the role of wave forces in structuring the fouling community

ABSTRACT

Wave forces have long been thought to serve as a controlling factor in the structure of intertidal fouling communities, however, quantitative information regarding the forces imposed on fouling organisms by breaking waves and the ability of organisms to withstand these forces is nearly non-existent. To remedy this situation we have designed a simple FM/FM telemetry system capable of measuring the directional components of the forces imposed on small animals (eg. barnacles and limpets) by individual waves. This apparatus has been used at two sites on Tatoosh Island, near Cape Flattery, Washington; both sites being fully exposed to the prevailing Pacific swells. A total of approximately 100 hours of force recordings have been obtained for two species (an acorn barnacle, Semibalanus cariosus, and a limpet, Collisella pelta) and for small plastic spheres used to standardize and calibrate the system. The adhesive tenacities of six common intertidal species have been measured. These data have been partially analyzed and a preliminary picture of the results can be drawn.

(a) Water velocities and accelerations at the rock surface are very high. The maximum values measured (with a wave height of approximately 4 m at breaking) were 8.5 m/s and 504 m/s². It can be estimated that during extreme storm conditions (wave height at breaking = 10 m) flow values of 14 m/s and greater than 1000m/s² may be reached.

(b) These flow conditions place considerable forces on fouling organisms adhering to the rock surface. Each species exhibits a distribution of adhesive tenacities and the probability that an organism may be dislodged during specific wave conditions can be calculated. For organisms with large tenacities and small drag, lift, and mass coefficients this probability may be relatively small; 15% for the acorn barnacle Balanus glandula (at flow conditions of 14 m/s and 1000 m/s²) while for other species with smaller tenacities and less stream lined shapes the probability of dislodgement is high; 72% for a solitary

mussel, Mytilus californianus.

(c) The stress (force/area) imposed by breaking waves on the adhesive system of intertidal organisms is, in part, a function of the size of the organism; the larger the animal the greater the stress and the greater the adhesive tenacity required to resist dislodgement. This dependence of stress on size is a result of the rapid water acceleration occurring during wave breaking, and for those species with an appreciable probability of dislodgement this factor may serve to limit the size to which these organisms can productively grow.

(d) Surprisingly, substantial lift forces (ie. forces perpendicular and away from the rock surface) are encountered by intertidal organisms during rapid water flow. For organisms with relatively large lift coefficients (C_L) (ie. the limpet (Collisella pelta, $C_L = 0.33$ at a Reynolds number of 1.10^5) the lift force exceeds the drag force. To a certain extent C_L can only be minimized by maximizing the drag coefficient, C_D , and the evolutionary design of shell shape in intertidal organisms may represent an attempted compromise between lift and drag forces.

(e) A negative correlation was found between wave force and the frequency with which that force was encountered. However no correlation was found between still water level and the time at which the maximum force was encountered during a particular tide; ie. an animal may be washed-over by fewer waves at low tide than high tide, but the probability of being hit by the maximum wave force is independent of the water level. These factors may influence the foraging strategies of mobile fouling organisms.

PLANS FOR THE FUTURE

This is the final year of this contract. Data analysis will be completed and publications prepared. Further work is planned on the design of the adhesive systems of intertidal organisms, and on the relationship between lift and drag coefficients.

CURRENT REPORTS AND PUBLICATIONS

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(b) M. W. Denny (1981), "A quantitative model for the adhesive locomotion of the terrestrial slug, Ariolimax columbianus." *J. exp. Biol.* 91: 195-217

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(d) M. W. Denny, T. Daniel and M. A. R. Koehl. "Wave forces and the limits to body size in intertidal organisms." (in preparation)

(e) M. W. Denny. "Wave forces on solitary intertidal organisms." (in preparation)

(e) M. W. Denny. "A simple recording dynamometer for measuring wave forces on intertidal organisms." (in preparation)

BIOEROSION OF LIMESTONES BY BORING SPONGES: AN EXAMINATION
OF PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF BORING

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ASSISTED BY

WORK UNIT NO. NR 104-960

CONTRACT N00014-79-C-0916

OBJECTIVES

(a) To examine the biochemical mechanism of boring by sponge etching cells; and (b) to determine the effects of environmental factors on rates of sponge boring.

ABSTRACT

Boring sponges are primary bioeroders of calcium carbonate substrates. They are most destructive in tropical environments where they are known to attack limestone-aggregate concrete structures. The process of sponge bioerosion is cellular, and involves partial chemical dissolution of substrate followed by mechanical displacement of etched chips of substrate by sponge cells.

Assays of whole sponge extracts by others have identified compounds unique to boring sponges. These compounds have been implicated in the dissolution of CaCO_3 , and include peptide alkaloids, phenolic aldehydes, fatty acids, and the enzyme carbonic anhydrase. To determine what role each of these compounds plays in the bioerosion of limestones, it is first necessary to examine their distribution among the various cell types in the sponge.

Sponge tissue is composed of a variety of morphologically and physiologically distinct cell types. Among these, there are 2 sub-populations of cells which are involved in bioerosion: the etching cells (differentiated amoebocytes which lie immediately adjacent to the CaCO_3 substrate); and "cells with inclusions" (at least 3 cell types which may be involved in the synthesis, storage, and transport of compounds involved in CaCO_3 dissolution).

Techniques have been developed for culturing sponge cells in control CaCO_3 substrates. Cells are then harvested from actively boring cultures and mechanically dissociated in calcium- and magnesium-free seawater. Following dissociation, cells are separated into various sub-populations using Ficoll density-gradient centrifugation techniques developed during the course of this project. The purity of separated fractions is checked by epifluorescence microscopy of acridine orange-stained sub-samples.

Lyophilized fractions are currently being analyzed for compounds implicated in the dissolution of CaCO_3 . Results to date show that the peptide alkaloids most

strongly implicated by others as etching compounds do not occur in separated etching cells and cells with inclusions. While this does not rule out the involvement of peptide alkaloids in biodeterioration of limestones by sponges, it suggests that: 1) the biochemical mechanism responsible for CaCO_3 dissolution may differ among species, or between geographically isolated populations of the same species; and 2) the isolation of unique compounds from whole sponge extracts may not necessarily mean that such compounds are responsible for CaCO_3 dissolution.

Studies of the effects of environmental factors on rates of boring by sponges were interrupted during August 1980, when Hurricane Allen hit the north coast of Jamaica. Storm waves over 30 ft. high created surges which overturned boulder-type corals and crushed coral branches down to 120 ft. All experimental cultures were washed away, but new cultures were established within one month of the hurricane. In addition, the hurricane damage provided a unique opportunity for monitoring recolonization of the newly available coral rubble substrate, thereby providing a natural control for field experiments. Observations have revealed that during the first 4 months following the hurricane, one species of boring sponge, Cliona caribbaea, out-competed all other borers. This species, which bores into both boulder-type corals and branching corals, had been one of the less abundant species; it now inhabits approximately 90-100% of the recently exposed substrate. Two factors could account for this tremendous success in competition for substrate: 1) exposure of new substrate may have coincided precisely with egg production and larval settlement. Since no life history studies of C. caribbaea have been made, this theory cannot be confirmed. 2) C. caribbaea could have spread out from the bored bases of branching colonies. This would imply an extremely rapid growth rate. It is most likely that a combination of these two factors contributed to the exclusive colonization of new substrate.

PLANS FOR FUTURE

Research efforts over the past several years have been directed towards an understanding of the mechanism of boring. The remaining questions center around stages in the life history of boring sponges, particularly with respect to settlement, substrate selection, periods of dormancy, and stimuli for resumption of boring following dormancy. Both the success and the vulnerability of an organism vary with the stages of its life history. This has been vividly demonstrated by the success and failure of several species of boring sponges in Jamaica following a natural control, Hurricane Allen. Life history studies will give a fuller understanding of the mechanisms which affect sponge bioerosion, and will highlight opportunities for external intervention and control of biodeterioration of CaCO_3 by sponges.

CURRENT REPORTS AND PUBLICATIONS

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(c) S. A. Pomponi. "Colonization of coral rubble at Discovery Bay, Jamaica by Cliona caribbaea following substrate destruction by Hurricane Allen." (In prep)

(d) S. A. Pomponi. "A photogrammetric method for measuring rates of sponge boring in vitro." (In prep)

(e) S. A. Pomponi. "The role of carbonic anhydrase in sponge boring and localization of its activity in vivo." (In prep)

(f) S. A. Pomponi. "Analyses of sponge etching cells and accessory cell types using cell sorting techniques." (In prep)

Experimental Investigations Linking Micro- and Macrofouling

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ASSISTED BY R. Dills

WORK UNIT NO. NR 083-012-30

CONTRACT N00014-80-C-0252

OBJECTIVES

To determine the interrelationships between molecular, microscopic and macroscopic fouling.

ABSTRACT

The funds awarded us were intended to be used for a dual purpose. First, we were encouraged to rewrite our original proposal in an expanded version, paying particular attention to the methods to be employed and the design of the 2nd and 3rd year experiments. This 35 page proposal was submitted to ONR early in 1981 and subsequently sent out for review. The remaining portion of the support awarded us was to be devoted to beginning our experimental program.

In preparation for the experimental portion of our program we obtained permission to utilize the facilities of the Sundquist Marine Laboratory at Anacortes, Washington. Equipment was ordered and received. Bacteria and diatoms were collected from the Anacortes area and tentative identifications made. Certain of these organisms were then maintained in laboratory cultures. These included the diatoms Nitzschia angularis, Amphora coffeaeformis and Stauroneis amphoroides. Growth curves were determined for the bacteria. The bryozoan, Bugula pacifica, which was to be our test macroorganism, was collected and kept in containers of seawater in a dark 12°C coldroom. Several group meetings were held in which we revised and discussed our experimental approach in light of the most recent literature and our discussions with other investigators. In light of the short term support allotted us, we have accomplished a considerable amount and regret that we will not be able to carry these experiments further at this time.

CURRENT REPORTS AND PUBLICATIONS

- (a) A. Schoener and C.H. Greene. Comparison between destructive and non-destructive sampling methods in fouling research. Limnology and Oceanography (in press).
- (b) A. Schoener and T.W. Schoener. The dynamics of the species-area relation in marine fouling systems: Biological correlates of changes in the species-area slope. American Naturalist (in press).
- (c) A. Schoener. Artificial substrates in marine environments. In: J. Cairns, Jr. Ed., Artificial Substrates in Aquatic Community Analyses. Ann Arbor Science Publ., Inc. (in press).
- (d) S. Ahmed and A.L. Devol. Are high rates of sulphate associated with anaerobic oxidation of methane? Nature (in press).
- (e) S. Ahmed and S.L. King. Comparative measurements of sulfate reduction by radioisotopic and enzymatic technique. Marine Chemistry (in press).
- (f) S. Ahmed, Q. Dortch, J.R. Clayton, Jr., S.S. Thoreson and S.L. Bressler. Response of marine phytoplankton to nitrogen starvation: decreased nitrate uptake vs. enhanced ammonium uptake. Marine Biology Letters (in press).
- (g) F.DeWalle and J.E. Ongerth. Pretreatment control of toxic substances discharged to municipal sewage treatment plants. J. Water Poll. Control Feder. 52: 2246 (1980).
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BIOLOGICAL STUDIES OF MARINE BORING AND FOULING MOLLUSKS

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WORK UNIT NO. NR 104-687

CONTRACT N00014-76-C-0281

OBJECTIVES

In this long-term project our interests continue to be centered around a) reproduction, larval nutrition and larval settlement of wood boring bivalves, b) ecological and distributional studies of the Pholadacea, particularly with reference to the maintenance of water front structures, c) deep-sea wood borers and the role of wood in deep-sea ecosystems, d) refinement of Pholadacean systematics based on findings resulting from studies in a, b, and c.

ABSTRACT

Our experimental work on brooding in Lyrodus pedicellatus has been supported by detailed morphological investigations revealing the mechanism of embryonic nutrition. SEM and TEM examination and histochemical analysis of the gills showed that cells of the interlamellar junctions are specialized for synthesis and produce large quantities of glycogen. Mature junctional cells full of glycogen are found free in the lumen of the brood pouch, and in the mouth, esophagus, stomach and digestive diverticulae of brooded embryos. These cells, a parentally derived source of nutrition, are sloughed off the walls of the brood pouch and are eaten by the embryos. This study, combined with our earlier work using microbalance weighing to demonstrate the presence of an extra-embryonic nutritional source and isotopic tracer techniques to show that it was parentally derived, constitutes the first evidence of parentally derived extra-embryonic nutrition within the marine Bivalvia. An examination of the extensive collection of preserved shipworms in the Museum of Comparative Zoology documented the brooding type of all but one of the 20 species of shipworms known or inferred to brood, thus doubling the number of brooders for which the life history is known. Combined with our extensive field and laboratory studies this demonstrates 1) that reproductive specialization extends from oviparity through short-term larviparity, synchronous long-term larviparity, and culminates in sequential long-term larviparity, 2) that this specialization is inversely proportional to adult and gonad size but directly proportional to pediveliger size, and 3) egg size is constant for all teredinids and does not increase in relation to the size of young at release as in other marine bivalves. The constancy of teredinid egg size is a reflection of the unique occurrence of extra-embryonic nutrition in the long-term larviparous teredinids.

In addition to our field work in Australia, New Guinea, Panama and Florida, recent ecological-systematic studies of wood borers in cooperation with John Cheng Siang Kok, Director of Forests, Kuching, Sarawak and with J.J. Ewald, Professor of Biology, Zulia University, Maricao, Venezuela have produced some interesting generalities. Both these areas, (i.e. the coast of Sarawak from Cape Datoe to Cape Baram and Tablazo Bay and Lake Maricao, Venezuela) are largely mangrove. The

predominant species are oviparous; the short-term larviparous species (4 in Sarawak and 1 in Venezuela) are rare and, regardless of salinities, restricted to the lower reaches of the estuaries. No long-term larviparous species were present. Our field observations indicate that the 11 known long-term brooders (7 synchronous, 4 sequential) occur in areas where circulation is good, the water clearer, and oxygen tension higher than in the sluggish mangrove areas. We hypothesize that the reasons for this are: 1) the brooding of larvae reduces the ability of the parent to circulate water through the gills and hence the efficiency of gas exchange and 2) the oxygen demands of the larvae put an additional stress on the parent. We further hypothesize that respiration in long-term brooders is even more restricted because the brooding period is longer and the larvae grow to a larger size before release. In sequential long-term brooders not only are embryos always present in the gills of mature animals but they are contained in closed pouches which occlude the water tubes, thus greatly reducing circulation and increasing the need for well oxygenated water. Though supported by our field observations, laboratory experiments must be completed to test this hypothesis. Such information will contribute to our ability to predict species occurrence.

Continuation of work on material from our permanent bottom stations in the Atlantic and from the Eastern Pacific thermal vents have further supported my hypothesis that growth and reproductive rates in the deep sea are related to food availability. Analysis of the organisms removed from the panels show that the most diverse and abundant of the "wood island" species are the polychaetes, and that as the "island" ages the greatest increase in species is among the predators.

PLANS FOR THE FUTURE

(a) continuation and synthesis of life history and systematic studies of Teredinidae and Xylophagainae (b) continuation of studies on deep-sea borers and the role of wood in deep-sea ecosystems (c) further studies comparing deep-sea communities surrounding "wood islands" and "thermal vents".

CURRENT REPORTS AND PUBLICATIONS

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(b) K.E.Hoagland and R.D.Turner (1980), Range extension of teredinids (shipworms) and polychaetes in the vicinity of a temperate-zone nuclear generating station. *Marine Biology* 58, 55-64.

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(d) R.D.Turner (1981), "Wood Islands" and "Thermal Vents" as centers of diverse communities in the deep-sea. *Biologia Morya* 1981 No. 1, 3-10 [in Russian with English summary].

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STUDIES ON THE MOLLUSCAN WOOD BORER BANKIA GOULDI

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WORK UNIT NO. NR NR 104-194

CONTRACT N00014-78-C-0294

OBJECTIVES

(a) To investigate growth of the marine wood borer Bankia gouldi as indicated by rates of boring, protein synthesis, and CaCO_3 deposition; (b) to define the major processes involved in formation of calcified tubes of bankia; and (c) to examine conditions which influence in vitro mineralization of decalcified skeletal material of fouling organisms and crystal formation in metastable CaCO_3 solutions.

ABSTRACT

Previously we reported that Bankia can utilize dissolved glycine at ambient estuarine concentrations but that this is not a major source for protein synthesis. However, radioactive glycine is a useful marker for synthesis of tissue protein and protein of the calcified lining of the burrow. Radioactive Ca can be used similarly for following calcium deposition.

Bankia removed from the wood, stripped of their calcified burrow linings, and placed in sea water will form a calcified tube similar to the natural burrow lining, as reported earlier. This has proved a valuable system for mineralization studies. Details of the sequence of events of tube formation have been observed by scanning electron microscopy:

1. Deposition of organic material over the outer mantle epithelium (0-3 hrs.).
2. Transition of loose organic material to an organic sheath (3-18 hrs.).
3. Deposition of patches of CaCO crystals in organic sheath (18-24 hrs.).
4. Continued deposition of CaCO_3 crystals resulting in a rigid calcified tube (24-48 hrs.).

The significance of these observations is that mineralization will occur only in the presence of specific organic material. This material has yet to be identified.

We have followed changes in ultrastructure of the tube and mantle epithelium, Ca uptake in mantle and tube, and patterns of organic deposition in the tube during tube development.

Ultrastructure. Individual crystals associated with strands of organic material increase in number and ultimately form alternating layers of prismatic and aggregated crystals. Mantle cells undergo changes commonly associated with synthesis of RNA and protein.

Ca uptake and deposition. Radioactive Ca taken up from sea water reached a steady state with the mantle within 2 hrs. At that time, approximately one half of the mantle Ca had exchanged with the medium, indicating two pools of Ca, as we previously reported. In determinations of rates of mineralization during tube development, it has been found that the maximum Ca deposition rate is associated with formation of the first prismatic layer and is considerably less before and after. Acetazolamide ($5 \times 10^{-4}M$), a carbonic anhydrase inhibitor, decreased the rate of Ca deposition more than 75%.

Deposition of organic matter in tubes. Both soluble and insoluble fractions of the organic matter of the tube were deposited continuously over a 6-day period, as indicated by radioactive glycine incorporation. Soluble organic material appears to be converted to the insoluble fraction, presumably as a result of cross-linking. Several fractions can be identified in the soluble material, one of which was present in greater amounts in tubes containing lower than average amounts of Ca. Further analyses of the soluble organic components are in progress.

In collaboration with Dr. C.S. Sikes, we have demonstrated that in metastable solution of $CaCO_3$ certain amino acids and their polymers influence crystal formation. For example, polyglutamic and polyaspartic acids, both with multiple negative groups, very strongly inhibit crystal formation and alter crystal form at $10^{-4}N$.

PLANS FOR THE FUTURE

(a) To complete biochemical analyses of the soluble matrix of the calcified tube of Bankia, particularly fractions associated with initiation of mineralization and decreased rates of deposition; and (b) to study effects of crystal poisons and organic compounds associated with skeletal material on (1) crystal formation in metastable $CaCO_3$ solutions and (2) on in vitro mineralization of decalcified organic matrix of barnacle shell, molluscan shell, and serpulid tubes.

PUBLICATIONS

(a) D.M. Manyak, 1981, "A device for the collection, and study of wood-boring molluscs: application to boring rates and boring movements of the shipworm Bankia gouldi". Estuaries. In press.

(b) D.M. Manyak and K.M. Wilbur, 1981, "Biochemical aspects of molluscan shell mineralization". Proceedings of the Symposium on Marine Biodeterioration, Bethesda, Md. In press.

LIFE HISTORY STUDIES OF FOULING BRYOZOANS

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ASSISTED BY Mary Sue Brancato and Christopher Gardner Reed

WORK UNIT NO. NR 104-985

CONTRACT N00014-78-0064

OBJECTIVES

Our objective is to build toward an understanding of the fundamental events and processes in the larval biology, settlement and metamorphosis of major species of fouling bryozoans. Special emphasis is placed on: (a) relationship between phototaxis, geotaxis and competency for metamorphosis; (b) role of specific properties of microbial films in settlement; (c) role of transported bacteria in affecting settlement by subsequent organisms; (d) cellular analysis of the mechanisms of early rapid morphogenetic movements in metamorphosis.

ABSTRACT

In spite of the importance of bryozoans as significant components of fouling communities, little is known about their reproduction and larval biology and, consequently, of how infestations are established. Our research focuses on the Cellularioida, a superfamily of anascan cheilostomes that contains genera (e.g., *Bugula*) with numerous species important in fouling communities throughout the world. This year's research was directed to three areas.

First, field studies were concluded on the occurrence of bacteria in certain species of *Bugula* and other cellularioids, continuing 13 years of data from California and four years of data from Woods Hole. Results document the consistent presence of gram-positive bacteria within the palial sinuses of certain species and the absence of these bacteria from larvae of closely related and sympatrically occurring species. The nature of this apparent symbiotic relationship is presently unknown, but is being investigated. Species transporting bacteria are distributed widely and, frequently, are the earliest bryozoans to appear in fouling communities. Because the bacteria are released as a cloud over the substrate during metamorphosis, they may affect substrate composition and alter subsequent settlement by other larvae. Bacterial symbionts have been successfully isolated from two species (in collaboration with Ralph Mitchell and Paul Boyle), and future studies are planned to assess the potential role(s) of these symbionts in community development.

Second, studies were completed of settlement by larvae of *B. simplex* and *B. stolonifera* on mixed natural microbial films. Tests were run in the laboratory and were conducted simultaneously on these sympatrically occurring bryozoans. These experiments document different preferences of the two species. Such findings caution against generalization about settlement even within congeners from the same locality.

Third, C.G. Reed has completed the first phase of detailed studies on the mechanisms that underly certain of the early rapid morphogenetic movements accompanying metamorphosis of B. neritina. Special attention has been directed to events in the first 60 seconds of metamorphosis. During this period, permanent attachment is accomplished, and a major reorganization of the larval body plan into that of the adult is begun. These studies document the existence of two mechanisms, previously unknown in B. neritina, that are easily and reversibly inhibited. The first involves reorientation of the wall region of the internal sac, which is necessary for fusion with the aboral epithelium. This movement is inhibited by cytochalasin B and, hence, is probably dependent on a microfilament-generated movement. The second involves deposition of the calyx and involution of the larval locomotory organ. Like Bowerbankia gracilis, these events are effected by reversal in direction of beat by cilia of the locomotory organ cells. The movements can be reversibly inhibited by KCl.

PLANS FOR FUTURE

(a) Identification of the factor(s) in microbial films that induce settlement by Bugula larvae (jointly with Mitchell lab); (b) determination of the potential role of transported bacteria in affecting the subsequent settlement by larvae of bryozoans and other species of macrofouling organisms (jointly with Mitchell lab); (c) experimental studies of the role of geotaxis in larval settlement; (d) ultrastructural and histochemical studies of the attachment between bryozoan larvae and substrate and similar studies of ancestrular body wall formation; (e) experimental and descriptive studies of the mechanisms underlying cataclysmic metamorphosis.

CURRENT REPORTS AND PUBLICATIONS

- (a) C. G. Reed (in press), "The role of ciliary reversal in the settlement of the marine bryozoan Bugula neritina." J. Cell Biol.
- (b) R. M. Woollacott (in press), "Association of bacteria with bryozoan larvae." Marine Biology
- (c) R. M. Woollacott (in press), "Environmental factors in bryozoan settlement." In: MARINE BIODETERIORATION AND FOULING (eds. J. D. Costlow and R. Tipper) U. S. Naval Institute, Annapolis

BIOTURBATION

MICROBIAL ACTIVITIES IN THE DEEP SEA BENTHIC BOUNDARY LAYER

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ASSISTED BY

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WORK UNIT NO. NR -83-004-80

CONTRACT N00014-79-C-0079

OBJECTIVES

The primary objective of this project is measuring rates of microbial decomposition of dissolved and solid organic substrates with simultaneous observations on oxygen depletion and sulfate reduction in the top sediments and the benthic boundary layer at depths of 4000 to 6000 m in the North and Central Atlantic. Concomitant fouling and corrosion processes are recorded and quantified. Automated amphipod traps spiked with radiolabeled food materials are used for studying rates of protein-carbon incorporation into various biochemical fractions of deep-sea scavengers as compared to those of their intestinal microflora.

ABSTRACT

We have developed a technique - based on the use of free vehicles - for gathering a critical mass of data of in situ measurements on the ocean floor. This approach eliminates decompression effects and, at the same time, extends our depth limit indefinitely in contrast to similar work done earlier with DSRV ALVIN. Most of our automated techniques were originally developed on the basis of experiences gained with the research submersible. During the 1980/81 research period we used three free vehicles with timer releases for up to four signals which controlled the automated operation of four different programs: (1) microbial transformations of radiolabeled substrates, (2) microbial fouling on various surfaces, (3) microbially mediated corrosion of metals within a redox potential gradient, and (4) trapping and incubation of amphipods in the presence of radiolabeled solid food sources for comparative studies on the metabolism of deep sea scavengers and their intestinal microflora. Deployments were done at depths of 3600 and 5330 m (R/V OCEANUS Cruise #92, leg 4, 8-20 March 1981, San Juan - Woods Hole). Two shallow water control deployments in Buzzards Bay were done at 12 m depth during fall of 1980 and spring of 1981. All deployments were successful. The radiolabeled bait material was prepared from the protein fraction of a ^{14}C -glutamate-grown bacterial culture. This new bait material - as compared to in vivo labeled fish meat - exhibits a very low solubility during incubation and eliminates the irreproducibility of counts experienced in earlier test experiments. The data for the programs (1) and (4) are now being prepared for publication. Earlier data were presented at the Annual Meeting of the American Society for Microbiology in Dallas on 3 March 1981.

PLANS FOR FUTURE

The next two cruises in our research schedule are: 30 October to 10 November 1981, for vehicle deployments at ca. 5000 m west of Bermuda. and 23 July to 5 August 1982 at ca. 3000 m north west of Newfoundland. This latter cruise is in line with our plan to study new types of sediments and bottom communities whenever possible. Our long-range plans concentrate on the complete microbial carbon and sulfur transformations in the top sediment layers and the metabolic interactions between epibenthic scavengers and their microbial gut flora. The deep sea corrosion and fouling studies will be dependent on future ONR funding.

CURRENT REPORTS AND PUBLICATIONS

- (a) H. W. Jannasch (1980), "Microbial decomposition of organic matter in the deep sea." In: Resistencia a los antibioticos y microbiologia marina. VI. Nat. Congr. Microbiol., Santiago di Compostela, Spain, pp. 145-157
- (b) C. O. Wirsen and H. W. Jannasch (1980), "Experiments on microbially mediated corrosion on the deep sea floor." 2nd Int. Ocean Dump. Symp., April 1980, Woods Hole, p. 41-42
- (c) D. M. Karl, C. O. Wirsen and H. W. Jannasch (1980), "Deep sea primary production at the Galapagos hydrothermal vents." Science 207, 1345-1347
- (d) H. W. Jannasch and C. O. Wirsen (1980) "Studies on the microbial turnover of organic substrates in deep sea sediments." In: Biogeochimie de la Matiere Organique a l'Interface Eau-Sediment Marine. R. Dumas (ed.), Actes des Colloques de C.N.R S. 293, 289-294
- (e) H. W. Jannasch, R. L. Cuhel, C. O. Wirsen and C. D. Taylor (1980), "An approach for in situ studies of deep sea amphipods and their microbial gut flora." Deep-Sea Res. 27, 867-872
- (f) C. O. Wirsen and H. W. Jannasch (1981), "Growth experiments on undecompressed natural and pure cultures of deep sea bacteria." Amer. Soc. Microbiol., Ann. Meetg., Dallas, March 1981 (Abstract)
- (g) H. W. Jannasch (1981), "Interactions between the carbon and sulfur cycles in the marine environment." In: Proc. SCOPE Workshop on Interactions of Biogeochemical Cycles, B. Bolin and R. B. Cook (eds.), Wiley, New York (In press)
- (h) H. W. Jannasch and C. O. Wirsen (1981), "Microbiology of the deep sea." In: Deep Sea Biology, G. T. Rowe (ed.), Vol. 8 of The Sea, Wiley, New York (In press)

ASPECTS OF THE BENTHIC SAMPLING
IN SUPPORT OF THE HEBBLE

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WORK UNIT NO. NR 083-231 CONTRACT N00014-75-C-0201

OBJECTIVES

(a) To assess the variability of the standing stock of the sediment-modifying animals from the HEBBLE site, and (b) to use these data to design the main HEBBLE biological sampling.

ABSTRACT

The High Energy Benthic Boundary Layer Experiment (HEBBLE) is an investigation into the dynamics of the benthic boundary layer in an area where near-bottom current velocities are high. HEBBLE's goal is to develop a model of sediment transport in the deep ocean. Sediment transport parameters include critical erosion velocity and hydrodynamic roughness length. Organisms alter the sediment fabric and surface in ways that affect these parameters. Deposit feeders ingest silt- and clay-sized particles, removing a portion of their microbial and organic coatings, and egesting them as sand-sized fecal pellets or casts. Casts are frequently deposited on the sediment surface. Fecal casts, animal tracks, tests, and tubes create microrelief. Burrowing animals appear to decrease compaction and increase the water content of the sediment. Animals add mucus to sediments in their feeding and locomotion; bacteria use mucus to bind to sediment particles. All of these processes are dynamic in that the effect on the sediment of any one organism is being reversed by others. Clearly, organisms alter the tendency of particles to stick to each other, their packaging, i.e., bulk density, the hydrodynamic roughness of the surface, and the compaction of the bed in ways that are relevant to sediment transport. As a result, HEBBLE has a two-phase biological program (1) the characterization of the biota of HEBBLE site and (2) the use of shallow-water analogs in laboratory flume experiments to assess the importance of the biota to HEBBLE parameters.

In order to design the sampling program for phase 1, we required preliminary estimates of the variability. We took 0.25-m² box core samples in the HEBBLE area in May 1980; two box cores were partitioned in situ into 25 100-cm² box subcores nine of which from each core were used for biology; three additional cores were sampled on deck using four 100-cm² subcores. All subcores, in turn, contained a 25-cm² subcore for bacteria and particle samples. From the 18 75-cm² X 10-cm deep samples from the first two cores, I am measuring biogenous surface structures, and sorting out the animals to provide initial estimates of functional group standing stocks and their spatial variability.

The results show that although the animal standing stocks are low compared to those found in shallow water, they are an order of magnitude higher than comparable samples from the deep North Pacific. The harpacticoid copepods are particularly interesting. In deep-sea localities, harpacticoids have a broad range of life styles and associated morphologies. However, the HEBBLE harpacticoid fauna appears to lack surface dwelling species. Further, many species have conspicuous adaptations for burrowing. The high near-bottom currents in this area provide a possible explanation for these observations.

PLANS FOR THE FUTURE

I plan (1) to finish sorting the preliminary samples, (2) to identify the crustaceans from them, (3) to determine the spatial variability of the fauna, (4) together with Yingst to use these data to design the major HEBBLE biological samples, and (5) to publish a description of the community from the HEBBLE site (with Yingst).

CURRENT REPORTS AND PUBLICATIONS

(a) D. Thistle (1979), "Harpacticoid copepods and biogenic structures: implications for deep-sea diversity maintenance." In: Ecological Processes in Coastal and Marine Systems. R.J. Livingston (ed.), Plenum: New York, pp. 217-231 (completed with partial ONR support).

(b) D. Thistle (1980), "The response of a harpacticoid copepod community to a small-scale natural disturbance." J. mar. Res., 38, 381-395.

(c) W.S. Ravenel and D. Thistle (1981), "The effect of sediment characteristics on the distribution of two subtidal harpacticoid copepod species." J. exp. mar. Biol. Ecol., 50, 289-301.

FACTORS AFFECTING SEA FLOOR SEDIMENT ERODABILITY AND MUCUS EXOPOLYMER CONTENT

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WORK UNIT NO. NR 083-231-10

CONTRACT N00014-75-C-0201

OBJECTIVES

Objectives are three-fold: (a) to refine the quantitative methodology for the uronic acids as a measurement of mucopolysaccharide binding exopolymers; (b) to study the biological factors regulating formation and destruction of the exopolymers; and (c) to relate sediment binding exopolymers with geotechnical measurements of sediment erodability.

ABSTRACT

A quantitative methodology for estimating the mucopolysaccharide binding extracellular polymers formed by the micro- and macro-benthic fauna have been developed. Uronic acids with minor exceptions are markers of extracellular polysaccharides. The method consists of estrifying the uronic acids in the extracellular polymers and then reducing the polymer bound uronic acid methyl esters to the corresponding alcohols with sodium borodeuteride. The reduction provides two benefits: (a) the extracellular polymers lose much of their adhesiveness and come off the sediments; and (b) the loss of carbonyl function allows ready hydrolysis of the polymer by mild acid. The conditions are so mild that other cellular and extracellular polymers are not hydrolyzed. (Polymers containing uronic acids resist hydrolysis). The resulting neutral alditols are reduced to alditols and peracetylated for separation by glass capillary gas-liquid chromatography. The proportion of each pentose, hexose or heptose that was dideuterated with the reduction (hence was a uronic acid in the polymer) can readily be determined by use of coupled mass spectral fragmentography. Recovery of bacterial and plant uronic acid containing gums added to sediments was quantitative and the reduction of the esters was complete. The sensitivity is to 0.1 nmoles.

We have tested this technique with various sediments that appear to be stabilized. We have shown that several marine sedimentary *Pseudomonas* bacteria form different polymers that are enriched in mannuronic, galacturonic and glucuronic acids. Maldanid bamboo worms form tubes that stabilize sediments and are enriched in these uronic acids. The deposit feeding enteropneust *Ptychodera* forms fecal mounds that are depauperate in meiofauna and in bacteria and micro-eukaryotes (determined by the fatty acid composition of the extracted lipids) but enriched in galacturonic acid containing polymers.

Extracellular polymer films are important in marine fouling and corrosion. The microfouling film formed on metal surfaces exposed to running seawater that are repeatedly cleaned to maintain a high heat transfer efficiency is progressively

enriched in extracellular polymers. The microfouling plaque formed on aluminum is much more tenacious and per mg more resistant to heat transfer than the film formed on titanium. The microfouling film formed on aluminum has a 4-fold higher ratio of galacturonic acid to galactose in the extracellular polymer than the film from titanium.

To directly test the relationship between sediment erodability and exopolymer content, cultures of Pseudomonas atlantica were manipulated nutritionally to synthesize extracellular polymers over a 3 order of magnitude range in fine sand (2.5 - 3.5 phi). In cooperation with A. Nowell, we will measure the critical erosion velocity of these sediments to document the increased erosion resistance caused by the exopolymer.

PLANS FOR FUTURE

These methods can be utilized to study how the formation and breakdown of the extracellular polymers in sediments are related to the biomass, metabolic activities, physiological status and community structure of the benthic micro- and macrobiota. Predictive indicators for sediment stability could be generated and tested. To begin these studies the correlation between formation of extracellular polysaccharide polymer by marine bacteria and their metabolic status as determined by adenylate energy charge and the homeostatic mechanisms that maintain energy charge will be examined. Another measure of metabolic status is the formation of poly beta-hydroxybutyric acid (PHB) by benthic bacteria. PHB is formed during conditions of unbalanced growth. The relationship of extracellular polymer formation to PHB will also be measured. These correlations will form the basis for a M.S. thesis for D.J.U.

CURRENT REPORTS AND PUBLICATIONS

(a) S. A. Fazio, D. J. Uhlinger, J. H. Parker, and D. C. White (1981), "Estimations of uronic acids as quantitative measures of extracellular polysaccharide polymers from environmental samples". Appl. Environ. Microbiol. Submitted, August 1981.

(b) D. C. White, and P. H. Benson (1982), "Determination of the biomass, physiological status, community structure and extracellular plaque of the microfouling film". U. S. Naval Institute Press, in press.

(c) D. C. White (1982), "Sensitive analytical methods for the measurement of biomass, metabolic activities, nutritional status and community structure of microbial films". J. Great Lakes Research, in press.

(d) D. J. Uhlinger (1981), "Factors affecting exopolymer production by marine Pseudomonas". Abstr. Amer. Soc. Microbiol. 1981: 204.

ORGANISM-SEDIMENT INTERACTIONS IN DEEP-SEAFLOOR DEPOSITS AND THEIR
RELATIONSHIP TO SEDIMENT TRANSPORT IN THE BENTHIC BOUNDARY LAYER

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WORK UNIT NO. NR 104 - 129

CONTRACT N00014-80-C-0037

OBJECTIVES

To understand animal-sediment interactions in the deep sea, particularly the role of benthic organisms in influencing the erodibility (alt. stability) of the seafloor and the genesis of bedforms.

ABSTRACT

We are particularly interested in the biological community in an area of high near-bottom current velocities on the Nova Scotian Rise in approximately 4650 m of water, site of the High Energy Benthic Boundary Layer Experiment (HEBBLE). Analysis of samples obtained during a cruise on the KNORR to this area in April 1980 are providing us with preliminary information regarding macro-, meio-, and microfaunal densities, patterns of distribution, and bacterial activity on several spatial scales; 2) functional groups of the species present; and 3) physical and chemical sedimentary characteristics. Data from continued analyses of faunal and sedimentologic samples taken from 0.25 m² box cores support our initial observation based on X-radiographs of vertical sediment slabs taken concurrently. Sediments of this region of the deep-seafloor appear to be alternately dominated by periods of physical disturbances by strong bottom currents resulting in sediment erosion, and periods of weaker currents when deposition and biogenic reworking occur. Extensive networks of tubes and burrows throughout regions of discrete sediment layers implies that physical sediment reworking by bottom currents is intermittent. In addition, little flocculent material was observed on the sediment surface even within small subunits of a vegetatized box core, supporting the idea of slow deposition mixed with periods of erosion.

Macro- and meiofauna are most abundant in the top 2 cm, however, most species appear to be burrowers or tube dwellers rather than epibenthic. The majority of the fauna appear to be surface deposit and suspension feeders rather than deep-feeding deposit feeders, as suggested by examination of the organisms and as evidenced by the absence of any aggregations of coarser grained particles into distinct layers at depth with the sediments. The make up of the faunal samples will be completed this fall and the results written up in collaboration with D. Thistle.

Sediment bacterial densities, ATP, and total adenylate concentrations are one to two orders of magnitude lower than in nearshore areas, but appear to be higher than in other deep sea regions at comparable depths. The presence of strong near bottom currents may help transport more utilizable organic material to HEBBLE area sediments than is available in other more tranquil regions. Organic carbon concentrations throughout the top 10 cm are twice as high, for example, as reported for Pacific Ocean areas. The availability of more organic material to the seafloor coupled with a downward mixing of this material into the sediment by biogenic reworking provides substrates for sediment microorganisms and may also help explain our observations that meio- and macrofauna are an order of magnitude more abundant than at comparable depths elsewhere.

In addition to the work up of samples obtained on the April 1980 cruise, we have been experimenting with methods to improve and optimize our extraction of ATP from marine sediments. We have completed this work and are in the process of writing up our findings (Yingst and Berman, in preparation).

PLANS FOR FUTURE

During the coming year, I plan to 1) complete the biological and sedimentological analyses in progress; 2) work with D. Thistle to identify the functional groups of animals from our HEBBLE area box cores and asses their abundance variability, and 3) relate particle size analyses and descriptions to the identification of biogenic structures in order to examine how animals modify the sediments. This information will be important for future laboratory flume experiments relating organism activity to sediment transportability.

I will also be preparing for a cruise to the HEBBLE site during the summer of 1982. D. Thistle and I plan to sample the selected site using transporter-navigated vegematized 0.25 m box cores.

CURRENT REPORTS AND PUBLICATIONS

a) J.Y Yingst and S.B. Berman, "A reevaluation of extraction procedures for measurement of ATP in marine sediments with a comparison of deep sea and near shore environments (in preparation)

b) J.Y. Yingst and R.C. Aller, Biological activity and associated sedimentary structures in HEBBLE area deposits (in preparation)

c) J.Y Yingst (1981) "Introduction to the biological community in the HEBBLE area. II. The relationship of microbiol distribution and activities to sedimentologic characteristics." Abst. for Fall AGU Meeting, San Francisco.

NOXIOUS MARINE ANIMALS

GUIDE TO SHARKS OF THE WORLD

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ASSISTED BY

WORK UNIT NO. NR 104-168

CONTRACT NO14-80-C-0282

OBJECTIVES

To prepare textual information and to select illustrated material for a Guide to Sharks of the World, which will include data on their identification, natural history, distribution, and human impact. The Guide will be in a three-hole, loose-leaf format, similar to the regional fisheries identification sheets published by the Food and Agriculture Organization of the United Nations for fishes and other marine animals.

ABSTRACT

As originally conceived the Guide would include identification sheets for all shark families, species sheets for about half or less (170) of the living species of sharks, and generic sheets for certain large families (Squalidae, Scyllorhinidae, Triakidae, and Carcharhinidae). Additionally, sheets were prepared for the eight orders of living sharks, which provide a convenient means to subdivide the thirty shark families into manageable blocks. These shorten the familial diagnoses since ordinal characters are not repeated in them, and make for short keys to families that are more easily handled than a single long key to all thirty families.

The family sheets in their final form include a common and scientific familial name, a short list of field marks for quick family identification, a diagnosis listing the familial characters, color, size, behavior, feeding habits and reproduction, distribution and habitat, danger to humans, interest to fisheries, similar families, a list of species, keys to genera, genera and species, or species only, and references in abbreviated form. Species sheets are being prepared with family names, common and scientific names of the species, field marks, diagnosis, color, size, similar species, behavior, feeding habits and reproduction, distribution and habitat, danger to humans, interest to fisheries, a world map showing distribution, and references. The genus sheets include family, common and scientific names, field marks, diagnosis, and optional space for additional data including keys to species.

The diagnoses in order, family, genus and species sheets are hierarchical; diagnostic information at each level is not repeated in the level below it to save space because of the number of species being dealt with. An innovation of the present versions of the order, family, genus and species sheets is the field marks section, which, when possible, indicates obvious characters that separate the taxon from other similar taxa. This section is not necessarily hierarchical in lower taxonomic categories than orders, and may include characters from higher levels if they provide an easy way to identify the taxon. The field marks section is slanted towards quick identification in the field, especially in the water.

For monotypic orders (Pristiophoriformes, Heterodontiformes, and Squatiniformes), separate family sheets were not prepared, but information normally presented in the family sheet was included under the order. For monotypic families species sheets were prepared only if the single species was of special interest to people for fisheries or other reasons. For polytypic families with no species of special interest the species are listed and keyed out in the family sheet, but no species sheets are presented. A few to most of the species are covered by species sheets in the remaining polytypic families.

Illustrations for the published version of the Guide will include lateral views of a representative species for the ordinal, familial, generic, and species sheets; ventral view of the head, representative teeth or tooth sets, and other structures when appropriate for the species sheets; illustrations for characters in all keys; and picture guides to orders and families.

Present work on the guide primarily includes completion of the species sheets.

PLANS FOR FUTURE

Completion of my written contributions to the Guide to Sharks of the World, in 1982.

CURRENT REPORTS AND PUBLICATIONS*

(a) L. J. V. Compagno (1981). FAO Sheets. Sharks. Fishing Area 34. Ca. 180 pp. Food and Agriculture Organization of the United Nations, Rome.

(b) L. J. V. Compagno (in preparation, 1981) FAO Sheets, Sharks. Fishing Area 51. Food and Agriculture Organization of the United Nations, Rome.

(c) L. J. V. Compagno (in preparation, 1981). FAO Species Catalogue. Vol. __. --Sharks of the World. Food and Agriculture Organization of the United Nations, Rome.

* Includes only items of special relevance to the Guide.

SHARK REPELLENTS: PROTOCOLS FOR A BEHAVIORAL BIOASSAY

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WORK UNIT NO. NR 083-060

CONTRACT N00014-80-C-0042

OBJECTIVES

- 1) Develop valid and reliable test procedures permitting the screening of relatively large numbers of substances for their shark-repellent qualities.
- 2) Adapt such new procedures to the lemon shark.
- 3) Use newly developed bioassays as tests to investigate the theory that detergent-like surfactants repel sharks.
- 4) Train a student under the ONR Science Education Program.

ABSTRACT

In the search for an effective chemical shark repellent, the toxic secretion of the Moses sole (Pardachirus marmoratus) seemed very promising. Pardaxin (PX) the derived poison, strongly inhibited feeding of captive and wild sharks (but the substance is difficult to collect, expensive and unstable). Thus studies in several laboratories were undertaken to determine the mode of action of PX in hopes of finding a less complex, stable analog. One group (Zlotkin and Barenhotz) suggested that PX's repellent qualities arose from its molecular structure. The protein PX possesses a charged configuration and a certain amino acid sequence providing for a strongly hydrophobic terminal with remainder of the molecule being hydrophilic. From this basic molecular structure arises PX's amphipatic, surfactant and detergent-like properties. Zlotkin suggested that these properties interfere with phospholipid membranes thus affecting sensory receptors and in turn repelling sharks. Therefore, any strong surfactant should repel sharks. It is this theory that we wished to test. Prior to this work, PX or its crude extract had been tested in the laboratory on three sharks: the reef white-tip Trianodon obesus; the Atlantic sharpnose Rhizoprionodon terra-novae and the spiny dogfish Squalus acanthias. These relatively qualitative tests involved attempts to either feed a shark the toxic fish or expose sharks to PX; then determine its effect (which was death in one case). We report here the development of three new systematic tests for use as a bioassay in shark repellency. 1. Fish lethality test: Pupfish (Fundulus heterocleitus) are exposed for periods of either 6 or 24 h to various toxic materials. Physiological death is the response recorded. 2. Shark feeding test: Hungry lemon sharks (Negaprion brevirostris) are offered a whole bait fish which has a syringe attached to it. As the shark attacks the bait and bites the head, material is released into the attacker's mouth; it's response is recorded. 3. Tonic immobility (TI): Lemon sharks are held in an inverted position to induce a transient relaxed state known as TI. During this state, test substances are introduced into the shark's buccal cavity. That concentration of material which terminates TI (i.e., animal rights itself) is recorded. Using these three

bioassays, eight different substances were tested. Seven were strong industrial detergents; the eighth was a crude extract of P. marmoratus (PMC) reconstituted with distilled water from a freeze-dried extract. PMC was the baseline material against which the other seven were compared. Results clearly demonstrated that simple, low molecular stable surfactants will repel sharks. While PMC was repellent in relatively low concentrations, sodium-dodecyl-sulfate-lauryl-sulfate repelled sharks at 1/10 the minimum effectual PMC concentration. Iso-octyl-phenoxy-polyethoxy ethanol was equally as effective as PMC in repelling sharks. The remaining five were either less effective or ineffective. Thus, we have developed behavioral bioassays using live sharks which confirm Zlotkin's theory that surfactants can be repellent to sharks. All three assays gave essentially the same result.

Under the ONR Science Education Program, Mr. R. Perez worked during the summer in the Florida Keys on a shark-tagging project in connection with life history studies on the lemon shark. Using a protocol including weighing, measuring, marking, sexing and injecting tetracycline for age validation, he captured and released 224 young lemon sharks.

Plans for the Future

Zlotkin and Gruber intend to seek funding for continued study and testing of potential chemical shark repellents from synthetic detergents. Possibly up to 100 different compounds will be tested. Any differential responding on the part of the sharks should provide clues to the physiological mechanism underlying the repellency. Elucidating this mechanism will be one of our primary goals.

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BIOLOGICAL SENSORS FOR THE DETECTION OF ELECTRIC, MAGNETIC, AND HYDRODYNAMIC FIELDS

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OBJECTIVES

(a) To reveal, measure, and interpret the sensory information provided by the oceans' electric, magnetic, and hydrodynamic fields; (b) to identify the pertinent receptor systems and to study their principles of operation; (c) to elucidate the importance of these sensory modalities in the animals' daily life; (d) to apply the biological findings to the development of new measurement techniques for oceanic research; (e) to protect men and underwater gear from damage inflicted by marine organisms, and to reduce human interference with ocean life.

ABSTRACT

(a) Marine sharks, skates, and rays are endowed with an acute dc and low-frequency electric sense enabling them to determine the precise position of their prey and to detect their drift in ocean currents. By the same token, they receive unambiguous electromagnetic information about their geomagnetic compass headings. The receptors for these stimuli are the ampullae of Lorenzini. (b) Most aquatic animals are extremely sensitive to minute water displacements in the low-frequency range (< 20 Hz). Their hydrodynamic sense plays an important role in the detection of biological and inanimate objects and may also serve to monitor and control their locomotory activity and to reduce drag. (c) Various kinds of sediment bacteria have permanent magnetic dipole moments resulting from intracellular strings of single-domain magnetite crystals. Their magnetic properties allow them to migrate in a constant direction despite the pronounced effects of thermal agitation in organisms of micrometer dimensions. Researches on these closely related topics are conducted from a combined physical-biological point of view, with emphasis on biological validity and physical rigor.

After completion of the dogfish experiments in Vineyard Sound, we extended our night-time studies on electrically evoked feeding attacks to the open-ocean blue shark, Prionace glauca, in 40 m of water, 25 km off Cape Cod. An odor source and two pairs of dipole electrodes were suspended at a depth of 5 m under the observation well of the project's fiberglass research vessel and dimly lit by underwater lights. The electrode pairs were positioned at distances of 30 cm on opposite sides of the odor source. Small amounts of herring chum were pumped through the odor port, while a prey-simulating dc dipole current of 8 microamp x 5 cm was passed between either the one or other pair of electrodes, with the inactivated pair as the control. As in the bottom-dwelling dogfishes, the blue sharks preferentially attacked the current-carrying electrodes, ignoring both the odor source and the control electrodes. Thus, despite the animals' keen sense of smell and their well-developed eyesight, these pelagic sharks entered the last, crucial stage of their attacks going by the prey-simulating fields.

Detectors of minute water displacements exist in the lateralis system of aquatic vertebrates and homologous hair-cell receptors of invertebrates. The biological significance of low-frequency hydrodynamic stimuli has, however, remained in controversy due to a lack of instrumentation capable of measuring and simulating naturally occurring displacement fields. We developed a system producing dipole displacement fields by feedback-controlled excursions of a rigid sphere of 1.25-cm radius. The system yields excitation amplitudes up to 1 mm in the frequency range from dc to 100 Hz. The near-field displacements thus produced may be described by a potential field for all but the lowest frequencies where the water viscosity complicates the physical picture. The boundaries of the finite test tank are accounted for numerically by a computer program based upon the theory of images. Fields generated by natural sources or by the excitation sphere are recorded with a second 1.25-cm radius sphere, suspended by a slender graphite-fiber truss with bridle. By choosing a neutrally bouyant sphere and adjusting the stiffness of the suspension bridle, the system could be tuned to achieve a frequency response flat within 3 db from 1 to 100 Hz, allowing reliable measurements of submicrometer fluid displacements. In preliminary experiments, the fish Gymnotus carapo executed obvious feeding responses with respect to periodic water displacements in the range from 1 to 5 Hz, while the copepod Centropages typicus escaped radially away from a one-cycle sine-wave stimulus, 1/30 sec in duration. These observations exemplify the importance of near-field displacement detection in the sensory repertoire of aquatic animals.

Also in progress are experiments on (a) the nature of the magnetic orientation of the stingray Urolophus halleri, (b) the temperature dependence of the behaviorally determined dipole moments of magnetic bacteria, and (c) the hydrodynamic fields of moving fish and their role in predator-prey detection.

PLANS FOR FUTURE

(a) To continue current research on the electric, magnetic, and hydrodynamic sensory world of marine organisms, (b) to analyze the biological receptor systems from an engineering point of view, (c) to integrate the findings with those of physical and chemical oceanographers, (d) to present the data in a format most accessible to those engaged in applied research and ocean management.

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FIELD INVESTIGATIONS OF SHARK BEHAVIOR

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OBJECTIVES

(a) To investigate the behavior of sharks in the natural environment - both remotely (via telemetry) and directly (via diver observation and cine/video) - in regard to diel rhythms of activity and movement, mechanisms of orientation and navigation, feeding patterns, and intra/interspecific social behaviors including aggressive interactions with humans, and (b) to develop the biotelemetry/recording instrumentation, diver hardware, study techniques, and data processing methods necessary for the above field studies.

ABSTRACT

In September 1981, field work in the Gulf of California was completed on A.P. Klimley's doctoral study of the schooling behavior of scalloped hammerhead sharks, Sphyrna lewini. Since July 1979, seven cruises (appx. 10 days each) were taken to offshore areas in the lower Gulf, six aboard the 55' Mexican research vessel JUAN DE DIOS BATIZ (CICIMAR, La Paz), and one aboard the 80' charter boat DON JOSE. Large schools of hammerheads (up to 225 indiv.) were observed and studied at four locations, Isla Las Animas, El Bajo seamount, Isla Cerralvo, and Gorda Bank. Additional work at the offshore sites, and at certain shark-fishing camps, was accomplished shore-based using a trailered 22' outboard skiff.

The research involved characterizing the shark schools in terms of size, seasonality of occurrence, and size, sex, and stereotyped behaviors of individuals. Ultrasonic telemetry and conventional tagging were used to determine diel and longer-term movement patterns. Captured individuals were examined for sexual maturity, food habits, etc. Because the sharks were shy of scuba, observations were made by free diving, with much behavioral data also videotaped using a self-contained underwater camera/recorder system. Shark sizes were measured in-situ using a twin-Nikonos stereophotographic system. Both the sonic transmitters and the color-coded dart tags were spear-applied underwater to free-swimming sharks.

Sixteen sharks were telemetered, some with depth-sensing transmitters. Of 13 individuals tracked at the primary study site (El Bajo), all showed a similar pattern of remaining in schools on the seamount during the day, then departing at dusk for the pelagic environment at least several km from the seamount. Both telemetry and tagging indicated that many of the sharks (but not all) return to the seamount the next day. One telemetered individual returned after being tracked to at least 9 km away the previous night. There is also evidence that some very deep dives occur during these nightly forays (pressure-crushed transmitters).

Analysis of the data should provide insights into why the sharks school. The

following functional hypotheses are being evaluated: (1) Reproduction, for proximity to facilitate courtship and copulation, (2) Refuging, grouping for protection during the inactive phase of the diel cycle, (3) Feeding, to increase predatory success through cooperation or more efficient foraging patterns, and (4) Migration, as an aid to navigation accuracy or efficiency.

In California, G. Pittenger has finished taking data for his masters study of the long-term movements, food habits, and age/growth of Pacific angel sharks, Squatina californica, at Catalina Island. The research involved conventional underwater tagging (400 sharks tagged, 75 resighted), ultrasonic telemetry (12 trackings, up to 4 mo.), and periodic sampling of individuals for gonad stages, stomach contents, and vertebrae for age/growth determination. On a time scale of months, the trackings revealed an interesting "semi-nomadic" pattern. The sharks tended to remain in limited areas for periods of several days to weeks, but then move relatively suddenly to new areas several km distant in one night. All sonic transmitters were applied at Ship Rock, the primary study site, but telemetered sharks eventually spread out to encircle the entire 75-km perimeter of the island.

Jeffrey Landesman is nearing completion of his study of horizontal and vertical movement patterns in blue sharks, Prionace glauca. Eighteen short-term trackings were conducted, with many sharks exhibiting an interesting periodic deep-diving behavior similar to that found by F. Carey (WHOI) in the Atlantic. Relatively rapid dives as deep as 400 m have been recorded, with descent/ascent rates of 30 m/min. A working hypothesis is that these dives are feeding related, and that their brevity is related to some adverse condition at depth, e.g., low oxygen.

PLANS FOR FUTURE

(a) To continue field studies of natural shark behavior, primarily on blue, mako, angel, and great white sharks in California, gray reef sharks in the tropical Indo-Pacific, and hammerhead sharks in the Gulf of California, and (b) to continue development, as needed, of shark-research hardware including ultrasonic transponders, radio-float transmitters, X-Y positioning systems, and unmanned telemetry monitoring devices.

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ON THE STRUCTURE AND ICHTHYOTOXICITY OF PARDAXIN

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OBJECTIVES

The structure and ichthyotoxic activity of Pardaxin--the principle active compound isolated from the Red Sea flatfish Pardachirus marmoratus.

ABSTRACT

The mechanism of the toxicity to fish of the toxin (named Pardaxin) isolated from the Red Sea flatfish Pardachirus marmoratus was studied in the isolated opercular epithelium of the teleost Fundulus heteroclitus. Pardaxin (80 µg/ml) administered to the short-circuited opercular epithelium produced remarkable electrical changes. Almost immediately a stimulation of the short-circuit current (SCC) was observed, which was accompanied by a rapid decrease in the transepithelial potential. Following this transient stimulation the SCC dropped to a value of about 15% of the original SCC. Identification of ion-fluxes involved in the effect of pardaxin was studied by ion-substitution of a specific ions and measurement of ion-fluxes. In Cl⁻ free Ringer, Pardaxin elicited a large stimulation of the SCC indicating no increase in the net Cl⁻ movement across the epithelium. Examination of the ²²Na⁺ and ³⁶Cl⁻ fluxes indicates the following. (1) Within 20 min. the sodium efflux was inhibited by 40.0%, causing a significant increase in the sodium influx (from the sea water side into the blood side); (2) the net

chloride secretion was inhibited by 48.8%. Since in this epithelium the net chloride secretion is in rate equal to the applied SCC, the inhibition of the chloride secretion may explain the inhibition of SCC caused by pardaxin. The increase in the net sodium influx however, could explain the observed transient increase in the SCC. In addition, pardaxin was shown to cause a remarkable increase in the permeability to urea and sodium in the elasmobranch Squalus acanthias in vivo. The sharks were positioned into a large apparatus with a rubber diaphragm separating the head from the rest of the body. Pardaxin (25 µg/ml) administered to the head section elicits a marked leak of urea and sodium down their chemical gradients. These studies show that in teleosts and elasmobranchs Pardaxin's action is by a disturbance of the fish ion regulation mechanism.

FUTURE PLANS

To continue the investigation on PX toxicity and mode of action.

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SECTION VI

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SECTION VI:
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